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Review paper

Drug–polymer conjugates: potential for improved chemotherapy

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Use of polymeric drug delivery systems is rapidly becoming an established approach for improvement of cancer chemotherapy. Zoladex®, a poly lactide-co-glycolide subcutaneous implant that delivers a luteinizing hormone releasing hormone analog over 28 days, is now the treatment of choice for prostate cancer, and a polyanhydride matrix containing BCNU is currently in phase III evaluation for treatment of glioma multiforme. Soluble polymers were first proposed as targetable drug carriers in the mid-1970s, and although the first conjugates are still at an early stage of development some, e.g. SMANCS (styrene maleic acid–neocarzinostatin) and monomethoxypolyethyleneglycolasparaginase, are now undergoing clinical evaluation and show considerable promise. Polymeric drug delivery systems are usually designed to produce an improved pharmacokinetic profile of an antitumor agent (controlled release) and in addition soluble carriers can achieve either first-order (organ specific) or second-order (tumor specific) drug targeting by virtue of the fact that they are usually administered intravenously and should theoretically access primary and secondary disease. Soluble polymeric carriers have the potential to improve the activity of conventional antitumor agents, peptide and protein drugs, and have recently been used in constructs for delivery of oligonucleotides. With increased awareness that the successful design of a polymeric drug delivery systems can only be achieved with prior consideration of the pathology and stage of the disease, tumor accessibility, biochemistry and cell biology of the target site, choice of appropriate therapeutic agent(s) and understanding of their fundamental mode of action, we have seen the emergence of a number of exciting and potentially more selective antitumor therapies based on polymer technologies. Here, the basic principles for design of soluble polymeric drug delivery systems are explained and illustrated using examples drawn from our studies on the development of *N*-(2-hydroxypropyl)methacrylamide copolymer conjugates for use in cancer chemotherapy. Those soluble polymeric carriers that are undergoing clinical evaluation are briefly reviewed.

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Introduction

The use of drug delivery in cancer chemotherapy has recently been comprehensively reviewed by Robert *et al.*[1] Growing interest in this field has been in part due to limited progress in the successful development of effective new drug entities, but also due to the realization that new approaches must be adopted if we are to see an improvement in therapeutic activity.[2] Many different systems have been explored: low molecular weight prodrugs,[3] macromolecular carriers (immunoconjugates,[4] natural polymers,[5] synthetic polymers[6]), vesicular or particulate systems (liposomes,[7,8] nanoparticles,[9] microparticles for regional therapy[10]), polymeric implants[11,12] and not least use of devices such as infusion pumps.[13] Several of these technologies have been tested clinically (infusion pumps are in routine use), but it is certainly true that many (including immunoconjugates and liposomes) have, as yet, failed to realize their promise in the clinic. This has been largely due to an underestimation of the difficulties that would be encountered (poor therapeutic activity often due to limited tumor accessibility and toxicity/immunogenicity of the delivery system itself) when transferring such complex technologies into man. Large-scale production of many drug delivery systems to acceptable pharmaceutical specification (including shelf life) has also proved difficult to achieve and often preliminary clinical investigations have been undertaken with poorly characterized materials.

Polymer-based drug delivery systems are thus far the least explored, but with the exception of the

medical devices, they have already shown the greatest clinical success. Zoladex® the poly lactide-co-glycolide subcutaneous implant delivering a luteinizing hormone releasing hormone (LHRH) analog over a 28-day period is the treatment of choice for prostate cancer[14] and a polyanhydride matrix containing BCNU is currently in phase III evaluation for treatment of glioma multiforme.[15] Soluble polymer conjugates comprising monomethoxypolyethyleneglycol (mPEG)[16] bound to various proteins including asparaginase and interleukin 2 (IL-2) have been tested clinically, and a mPEG–asparaginase conjugate awaits regulatory approval.

Another polymer–protein conjugate, styrene maleic acid (SMA)–neocarzinostatin (NCS), designated SMANCS, developed by Maeda and colleagues in Japan has been evaluated in more than 500 patients presenting with primary hepatoma or secondary tumours of the liver, and first results show an exceptional response rate and a marked increase in survival.[17] Polymers afford several advantages as drug delivery systems, but in particular synthetic polymers are already in daily use in many different biomedical applications, including prostheses, contact lenses, plasma expanders, wound dressings and pharmaceutical excipients (reviewed in Gebelein[18]), and therefore there is already a wealth of knowledge concerning their biocompatibility (toxicology) and possibilities for commercial production to specifications that would prove acceptable to regulatory authorities. Use of polymer-based technologies for improved delivery of cancer chemotherapy has not yet been as widely explored as the liposomal- and antibody-based strategies, so here the specific characteristics of soluble polymeric drug carriers will be explained, and the general principles relating to design of all macromolecular drug carrier systems outlined. Of course the characteristics which govern tumor localization of natural macromolecular carriers such as immunoconjugates also relate directly to the design of synthetic polymeric drug carriers. The latter have the added advantage that their structure can be tailor-made to optimize features such as molecular weight and inclusion of recognition moieties.

General principles

Polymer related

Helmut Ringsdorf first proposed soluble polymers as drug carriers in the mid-1970s with a paradigm for design of the optimal system[19] (Figure 1). He suggested that an ideal polymeric carrier would be hydrophilic to ensure water solubility (indeed the many effective carriers have been shown to solubilize poorly water soluble antitumor agents) and also contain the functional groups necessary to permit covalent linkage to the drug. The concept of covalent conjugation via a biodegradable spacer amenable to specific enzymatic or hydrolytic cleavage was proposed to afford the opportunity to design a macromolecular prodrug preprogrammed to deliver compound at the desired rate within the target compartment. To achieve specific localization Ringsdorf suggested that a targeting moiety may additionally be bound to the polymer to promote cell-specific uptake by receptor-mediated pinocytosis. A wide variety of polymers have since been explored as drug carriers for use in delivery of antitumor agents and a representative list is given in Table 1. Most polymers are initially selected as they are known to be inert in the body, but biologically active polymers have also been explored both as drug carriers and as antitumor agents in their own right (reviewed in Seymour[45]). Although many polymers (many more than shown in Table 1) and their conjugates have been reported as 'interesting' in *in vitro* tests, most have either not been screened *in vivo*, or do not confirm their potential during *in vivo* examination. Lack of improved therapeutic index *in vivo* has been common and additional polymer-related toxicity has frequently been reported.[30,35] Of all the polymeric carriers described in the literature, dex-tran, mPEG, *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymers and poly(aspartic acid) have been most

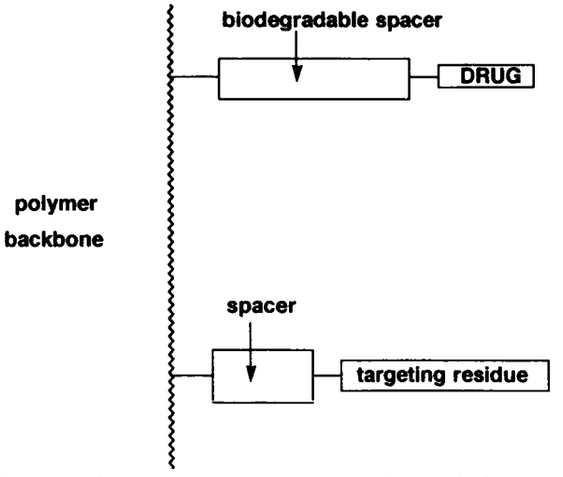


Figure 1. Schematic diagram to show the basic features of a soluble polymeric drug carrier.

Table 1. Soluble polymers synthesized as drug carriers for antitumor agents that have undergone biological/clinical evaluation

Polymer	Drug conjugated protein	Method of evaluation of antitumor activity	Comments	Reference
Carriers for conventional chemotherapy				
Dextran	Daunomycin	Tested *in vivo* in mice with YAC Moloney virus induced lymphoma for comparison with drug–dextran–antibody conjugates	LD_{50} was approximately 3 times greater than seen for free drug and conjugates could completely prevent tumor development	20
	Doxorubicin	Anti-human T cell monoclonal (T101) linked to doxorubicin via a dextran bridge tested against Molt-4 subcutaneous xenograft in BALB/c Nu/Nu mice	All *in vitro* treatments were superior to placebo, but a mixture T101 + Dox was more effective than T101-Dox-	21
	Mitomycin C	Preliminary clinical evaluation	Tumor responses, transient fever but no serious side effects	22
Polygalacturonic acid and carboxy-methylated yeast β-D-glucan	Ara C	Evaluated *in vivo* against L1210	ILS of conjugates was 89–166% compared with free drug 25%	23
N-(2-hydroxy-propyl)methacryl-amide copolymers	Doxorubicin	Evaluated against a number of animal and human tumor models *in vivo* including: L1210, P388, Walker sarcoma, M5076, LS174T xenograft, B16 melanoma	Antitumor activity seen in all models, T/C up to 762 seen against L1210 with long-term survivors	24
	Daunomycin	Administered intraperi-toneally against intra-peritoneal L1210 *in vivo* using a number of dosing regimes	Polymer conjugates consistently produced higher T/C than free drug and long-term survivors when administered at equi-dose	25
	Melphalan	*In vivo* evaluation against L1210 (intraperitoneal), B16 melanoma (intra-peritoneal), Walker sarcoma (subcutaneous)	Less effective than melphalan against intraperitoneal B16 melanoma, but more effective than free drug against Walker sarcoma	26
N-(2-hydroxy-propyl)methacryl-amide copolymers	Chorin e_6	Preliminary studies against mouse splenocytes and Alexander cells	Targeted conjugates were more active than non-targeted ones and less non-specifically cytotoxic than chlorin e_6	27
N-(2-hydroxy-ethyl)methacrylate–vinylpyrrolidone copolymer	Daunomycin	Drug bound via a hydrazone linkage designed for hydrolytic release; tested against L1210 *in vitro* and *in vivo*	*In vitro* and *in vivo* activity was reported	28

(*continued*)

Table 1. *Continued*

Polymer	Drug conjugated protein	Method of evaluation of antitumor activity	Comments	Reference
Poly(-L-glutamic acid)	Doxorubicin	Tested against L1210 and B16 melanoma *in vitro*	ID_{50} of conjugates was much lower than seen for free drug, but this was not surprising considering their different mechanisms of cell penetration	29
Poly(hydroxyethyl-L-glutamine)	Doxorubicin	Tested *in vivo* against MOPS 406, P388 and hemoblastosis La	No change in therapeutic index was seen for drug in conjugate form; the polymer induced hepatotoxicity in rats	30
Poly(α-malic acid)	5-Fluorouracil	Conjugates tested *in vivo* against intraperitoneal P388	Specific conjugates showed no acute toxicity at doses 200–800 mg/kg and were slightly more active than 5-fluorouracil, T/C ranging from 160 to 170%	31
Poly(aspartic acid)–PEG copolymers	Doxorubicin	Tested against five solid tumors *in vivo*, C26, C38, M5076, MKN-45 and MX1, and also intraperitoneal P388	Using the solid tumor models higher antitumor activity was seen for the conjugate seen for doxorubicin in most systems and toxicity of drug was reduced 20-fold; against P388 the maximum T/C was approximately 500	32, 33
Poly-L-lysine	Methotrexate	Pharmacokinetics and antitumor activity against L1210 in the pleural cavity of BDF_1 mice	The conjugate was more toxic than free drug due to poly-L-lysine-related toxicity; no change in the maximum ILS was seen, being about 30% for both at optimal dose	34
	Daunorubicin	Comparison of activity of poly-L-aspartic acid and poly-L-lysine conjugates using P388 and Gross leukaemia (intraperitoneal)	Drug conjugation to poly-L-lysine led to a reduction in both drug and polymer-related toxicity. Conjugates showed lower activity than free drug against Gross, but not P388. However, only the poly-L-aspartic acid conjugate produced an increase in activity above that of daunorubicin	35
	Oligonucleotides	Galactose modified asialooros omucoid bound to poly-L-lysine complexed with the plasmid pSV2CAT injected intravenously	CAT expression measured in the liver	36

(continued)

Table 1. *Continued*

Polymer	Drug conjugated protein	Method of evaluation of antitumor activity	Comments	Reference
Block copolymers of poly(ethyleneimine) and palmitic acid	Cyclophosphamide derivatives	Probably the first block copolymer system designed to deliver an antitumor agent; kinetics of release *in vitro*	Although detailed pharmacological data were not produced this important study showed ability to control rate and extent of drug liberated by controlling amount of palmitic acid and length/hydrophobicity of the spacer	37
Polymeric carriers known to exhibit inherent antitumor activity				
DIVEMA	Methotrexate	Inhibition of dihydrofolate reductase *in vitro* and antitumor activity against L1210 *in vivo*	The polymer conjugate showed an ability to inhibit enzyme and produced an ILS greater than methotrexate with 10–30% long-term survivors	35
	Doxorubicin	Tested against human ovarian cancer IGROV-1 in Swiss Nu/Nu mice (intraperitoneal)	Free doxorubicin was effective when given intraperitoneally but not when given systematically; conjugate was less toxic than free doxorubicin when given intraperitoneally and showed maintained antitumor activity	39
	Cyclophosphamide	Sulfydryl derivatives of 4-hydroxycyclophosphamide bound to DIVEMA and tested against L1210 *in vivo*	Optimal antitumor activity of polymeric derivatives occurred at lower doses than monomeric derivatives due to the conjugate; antitumor activity was comparable to cyclophosphamide	40
Polymer–protein conjugates				
Styrene–maleic anhydride	Neocarzinostatin	Intra-arterial administration of SMANCS in lipiodal to patients with hepatocellular carcinoma	Most tumors reduce to more than 50% of original size after 6 months	41
Polyethyleneglycol	Asparaginase	Phase II investigation in treatment of non-Hodgkin's lymphoma	mPEG–asparaginase given intramuscularly 2000 units/m^2 every 2 weeks; partial response in two out of 21 patients	42
	IL-2	Pharmacological activity *in vivo*	At equi-toxic doses PEG–IL-2 was more active	43
	G-CSF	Pharmacokinetics in rats	Slower plasma clearance resulted in prolonged increase in peripheral blood neutrophils	44

consistently reported as non-toxic following their broad evaluation in numerous test systems.

Polymeric carriers can be divided into two categories, truly synthetic, such as HPMA copolymers and divinylether maleic anhydride (DIVEMA), or natural (sometimes pseudosynthetic) polymers, such as polyamino acids and polysaccharides (dextran). Several polymers already have regulatory approval for use in pharmaceutical formulations including mPEG, cellulose esters, polyacrylic acids, poloxamers and dextran. Natural polymers, including polysaccharides and polyaminoacids, are often degradable thus facilitating metabolic removal from the organism after administration, and this can be advantageous as it prevents accumulation in the body. However, covalent conjugation of pendant groups has been shown to reduce the susceptibility of normally biodegradable polymers to enzymatic attack, e.g. modification of dextran reduces its rate of enzymatic hydrolysis by dextranases.[46] Degradable natural polymers are also frequently immunogenic[47] so their relative merits must be considered on a case by case basis. In contrast, man-made synthetic polymers are frequently non-degradable and therefore their clinical use must be restricted to conjugates of molecular weight lower than the renal threshold which can be readily excreted. Of course it will be very difficult to identify a polymeric carrier, be it natural or synthetic, which is ideal in every respect, but there are several criteria which should be met if a polymeric drug carrier is to function effectively, and these can be summarized as follows:

- Use of the carrier must not induce any additional toxicological burden, the carrier must not be immunogenic, and be effectively metabolized/excreted from the body
- The carrier should have sufficient drug carrying capacity to ensure therapeutic activity and if the conjugate is developed for targeting the bound drug must not interfere with the biodistribution of the conjugate
- The carrier must be amenable to production on a commercial scale, at acceptable cost, and must yield a well characterized, stable formulation which can be manufactured reproducibly and can be conveniently administered to patients.

Drug related

Drug delivery systems usually seek both to optimize drug pharmacokinetics (*controlled release*) and improve localization of drug in the tumor (*drug targeting*). It is important to realize the separate contribution of pharmacokinetic manipulation and targeting, and also acknowledge that in practice targeting of a substantial proportion of administered dose to tumor has rarely been achieved. These factors are distinguished schematically in Figure 2. Bolus administration of an antitumor agent typically leads to high peak concentrations in plasma, tumor and unfortunately also sites of toxicity—it being accepted that toxicity is predominantly determined by drug pharmacokinetics.[48] Controlled release formulations (Figure 2a) can be designed to reduce the peak plasma concentration and in theory prolong exposure to an effective drug concentration,[49] leading to both a reduction in toxicity and also increased efficacy—many antitumor agents express differential activity throughout the cell cycle, even though they are rarely stage specific.[50]

To design an ideal drug release profile it is necessary to consider the *in vitro* cell survival response curve (Figure 3) for the individual antitumor agent under consideration. If exposure of tumor cells to drug results in a biphasic or plateau response, prolonged release of a high dose of the compound *in vivo* would not be advantageous unless it facilitated increased drug penetration into a large solid tumor. Any pharmacokinetic change may also potentiate toxicity so design of an optimum profile must address the potential toxicological/therapeutic response for that drug. Maintained low level exposure of tumor cells to a cytotoxic drug may also induce resistance, so continued exposure must be viewed with caution.

Potency of the agent to be delivered and the clinical dose must also be considered at an early stage. One of the most important limiting factors when designing a polymeric drug delivery system is its drug carrying capacity, not only in terms of the theoretical possibilities for chemical conjugation, but also practical issues such as water solubility of the product and influence of high drug substitution on the pattern of biodistribution of the conjugate. Experience tells us that maximum drug loading does not always yield a product with maximum efficacy! Realizing that a relatively small proportion of the dose administered may reach the tumor, use of polymeric carriers to carry drugs such as 5-fluorouracil and methotrexate which are routinely administered to patients in high doses (up to 500 mg/kg) seems a concept fraught with problems. For this reason many investigators have chosen to combine particularly potent drugs with soluble polymeric carriers, e.g. anthracyclines, mitomycin C and *cis*-platinum analogs (Table 1).

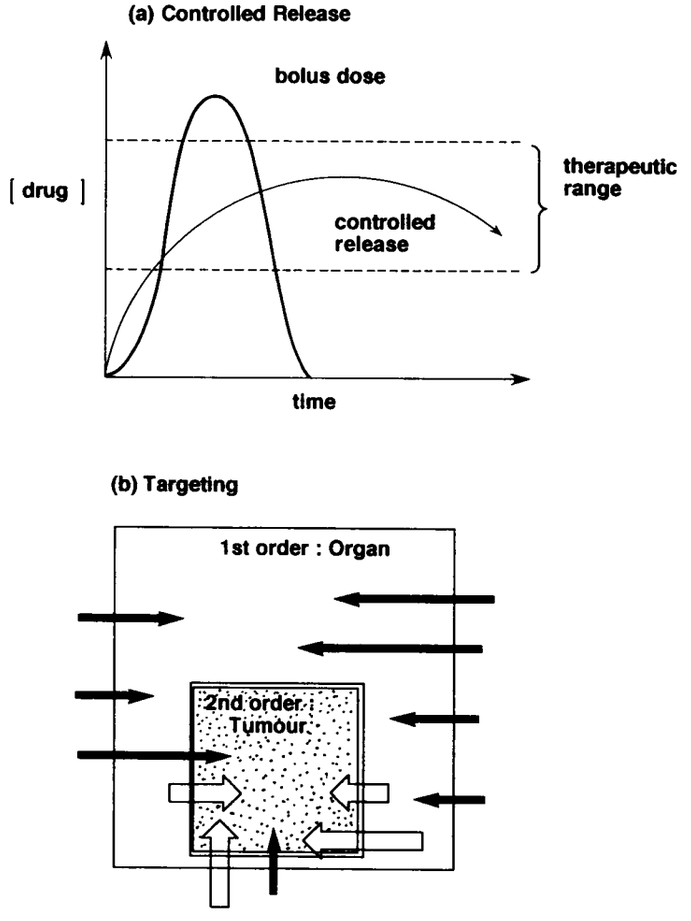


Figure 2. Theoretical aspects of (a) controlled release, and (b) first- and second-order drug targeting.

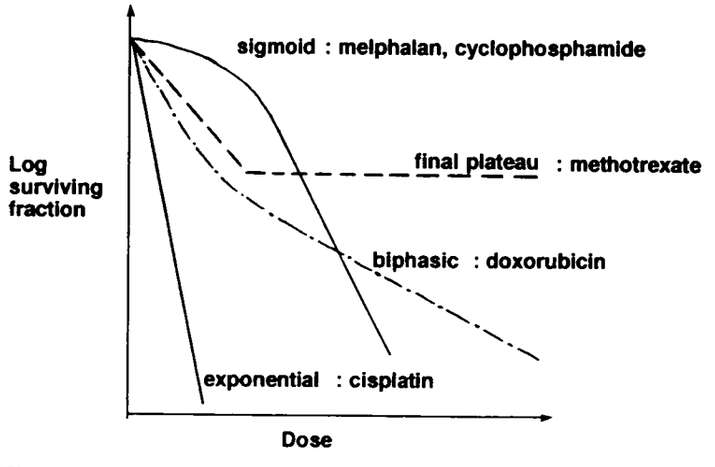


Figure 3. Cell survival curves after exposure to different antitumor agents (after Mauro *et al.*[50]).

The latter are particularly difficult to conjugate covalently, but binding to polymers can be achieved by non-covalent complexation.

Finally, it is important to consider the likely whole body and cellular fate of a drug prior to design of the carrying system. In the past, drugs which require access to an intracellular pharmacological receptor to initiate activity have been mistakenly bound to macromolecular carriers via non-biodegradable linkers, thus prohibiting drug liberation and therefore cellular penetration. Optimization of design can easily overcome this problem. Similarly compounds with limited plasma stability, e.g. some alkylating agents, would not seem good candidates for polymer conjugation as they should theoretically be inactivated whilst in transit before reaching their cellular target, unless of course the carrier could be designed in some fashion to stabilize the bound drug and thus limit hydrolytic degradation. If the macromolecular carrier enters the cell via the pinocytic route, the question of drug stability within the endosomal/lysosomal compartments must be carefully addressed before proceeding. Any compound which is acid-labile or subject to lysosomal degradation is not a good candidate.

Drug targeting using the macromolecular approach

The concept of the 'magic bullet' pioneered by Erhlich[51] may still be an unrealized dream in the context of specific delivery of cancer chemotherapy to tumor cells; however, significant advances have been made with the identification of many delivery systems that achieve very effective organ/compartmental (first-order) drug targeting (Figure 2b). Liposomes, particulate systems and macromolecular carriers have been developed which can deposit a large percentage of an intravenously administered dose into the liver or lung (examples are shown in Table 2). This approach holds promise for regional delivery of antitumor agents for treatment of primary or secondary disease, but clinical success is awaited.

To achieve tumor-specific (second-order) targeting it is necessary to identify unique features of tumor cell biology that will concentrate drug within the tumor. Most approaches have sought to produce monoclonal antibodies that will interact preferentially with tumor cell surface antigens (reviewed in Magerstadt[59]). Although tumor cells do express tumor-enhanced, or specific-antigens this strategy has not yet proved clinically successful for directed therapy probably due to: limited tumor access of these relatively large macromolecules, tumor cell heterogeneity, and the human anti-mouse antibody response seen in patients. Even when using antibodies of the highest affinity and specificity a relatively small fraction of administered dose is delivered to the tumor *in vivo* (possibly less than 0.1% dose administered in man[60]), but it is encouraging that this relatively small localization can theoretically be put to good use, exemplified by the antibody-directed enzyme prodrug (ADEPT) approach.[61] A spectrum of other cell surface receptors have been proposed as candidates for tumor selective targeting and examples of those explored experimentally are listed in Table 3, and discussed later. Soluble polymeric carriers can be synthesized to include many of the ligands proposed

Table 2. Drug delivery systems which achieve first-order (organ specific) targeting

Delivery systems	Organ of localization	Percentage of administered dose	Reference
Liposomes			
SM/CH (2:1 mol/mol) large multilameller vesicles	liver	84% (1 h)	52
SM/CH (2:1 mol/mol) small unilameller vesicles	liver	56% (24 h)	53
Mab 273-34A-liposomes	lung	54% (15 min)	54
Microspheres			
Polystyrene (15.8 μM)	lung	89% (11 days)	55
Polystyrene (1.27 μM)	liver	90% (11 days)	55
DEAE microspheres (40–160 μM)	lung	93% (11 days)	55
Soluble polymers			
HPMA–galactose–doxorubicin	liver	60% (5 h)	56
Polyamino acid (Glu–Ala–Tyr)	lung	80% (15 min)	57
Dextran–galactose	liver	71% (30 min)	58

Table 3. Tumor markers proposed as targets for second-order (tumor selective) drug delivery

Type	Comments
Receptors involved in constitutive biochemical pathways	
Tranferrin receptors	The surface density of transferin receptors has been correlated with the degree of malignancy and proposed as a tumor selective target (e.g. refs 62 and 63). However, the broad cellular distribution of this receptor has prevented fruitful use for drug delivery.
LDL receptors	Cells require cholesterol for membrane biosynthesis and most cholesterol is derived via receptor-mediated pinocytosis, the numbers of LDL receptors being subject to up- or down-regulation according to need (reviewed in ref. 64). It appears that human tumor cells have a higher density of LDL receptors than normal cells, but nonetheless the broad distribution of these receptors will never permit absolute specificity. However, it has been suggested that it is possible to down-regulate the number of LDL receptors on normal cells by diet.
Growth factor receptors	Many tumors have been reported to overexpress receptors for growth factors such as epidermal growth factor (EGF)[65] and fibroblast growth factor (FGF)[66]. The EGF receptor shows sequence homology with the c-*erbB*$_2$ oncogene product and is over-expressed in 20–30% of breast cancers.
Melanocyte stimulating hormone (MSH)	Melanocytes and malignant melanoma have a receptor which recognizes the peptide hormone MSH. Binding of MSH increases the levels of intracellular cAMP and stimulates tyrosinase activity, in the course of melanin production. Because of the relative selectivity this receptor has been used as a target for MSH–toxin constructs, antibody conjugates and other ligands.[67]
Cellular adhesion/recognition systems	
	Several aspects of cellular recognition and adhesion have been proposed as targets for chemotherapy, including laminin and fibronectin receptors.

for first- or second-order targeting, thus it is important to keep a broad awareness of any possibilities that promise tumor selectivity.

The principal consequence of drug conjugation to a macromolecular carrier (via a covalent linkage that is not rapidly hydrolyzed) is the limitation of the cellular uptake of drug to the mechanism of pinocytosis.[68] At the cellular level this contrasts with the ability of most low molecular weight antitumor agents to enter the cell by traversing (either actively or passively) the cell membrane before exerting their pharmacological effect. This is the single most important factor in determining altered pharmacological activity of all macromolecular drug conjugates irrespective of their composition.

Pinocytic internalization involves membrane invagination with concomitant capture of macromolecules (either in the occluded extracellular fluid or membrane bound), followed by immediate transfer into the endosomal compartment of the cell where many ligands and their receptors are known to dissociate owing to the acidic milieu[69] (Figure 4). Most macromolecules are then directed, via a series of vesicle fusion events, into a secondary lysosomal compartment ensuring continued exposure to an acidic environment (pH 5.0–6.0), but additionally to lysosomal enzymes which are capable of degrading every class of natural macromolecule entering the cell.[70] Like the plasma membrane, the lysosomal membrane is a natural barrier to macromolecular transfer and thus will only allow escape into the cytoplasm of low molecular weight products released as a consequence of lysosomal degradation.[71]

It is of course the restriction of drug uptake to the lysosomotropic route that allows exploitation of the opportunities for both passive and active targeting of drug to tumors.[72] The mechanism of pinocytosis can be divided into three categories. Fluid phase pinocytosis is a constitutive phenomenon common to all cell types, characterized by ongoing membrane invagination and capture of macromolecules present in the extracellular fluid. Uptake is poorly efficient and the rate is directly proportional to the extracellular concentration of the macromolecule. It has been suggested that certain tumors have a high basal level of fluid phase pinocytosis, but this has proved difficult to substantiate *in vitro* or *in vivo*. However, there is now unequivocal evidence that many different macromolecules, either natural molecules present in the circulation (e.g. albumin) or those administered intravenously, do passively accumulate (*passive*

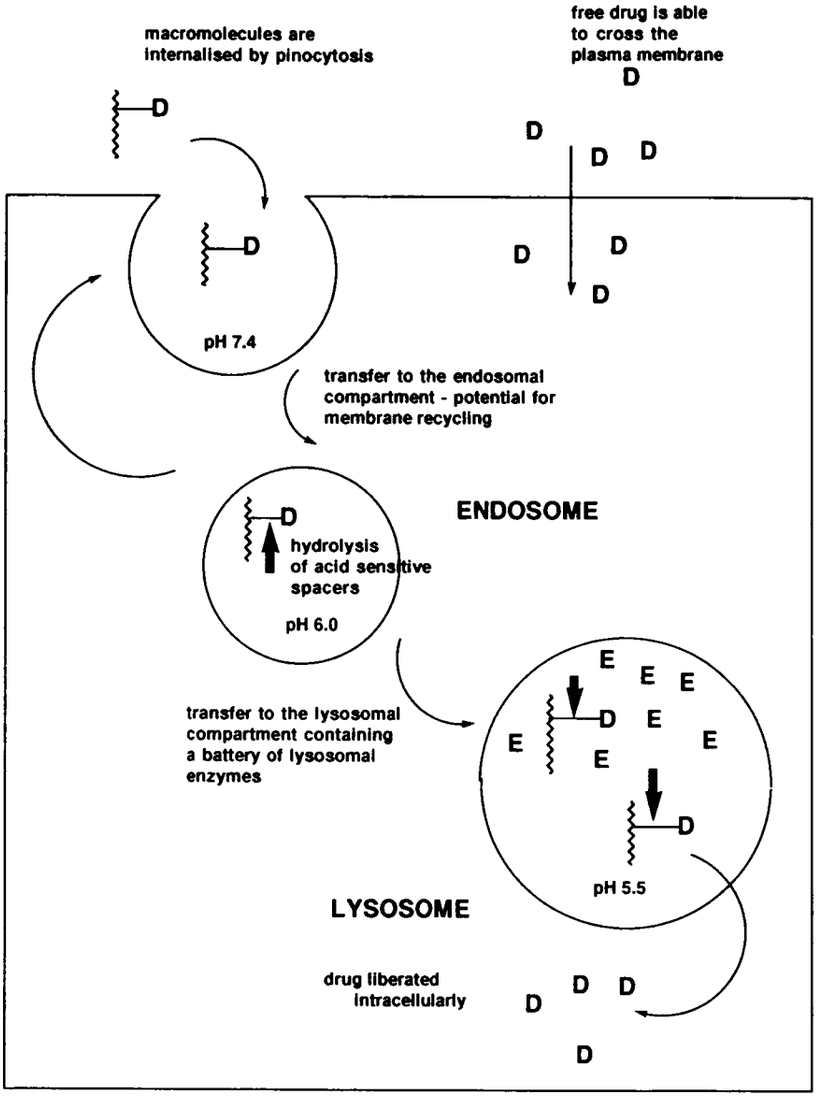


Figure 4. Comparison of the mechanism of cellular uptake of free drug (D) and polymer–drug conjugates (}-D). Following pinocytic internalization the macromolecular conjugate is transferred to the lysosomal compartment where it is exposed to an array of lysosomal enzymes (E).

targeting) within solid tumors. Over the last decade tumor accumulation of antibodies,[73,74] polymers[75] and polymer–protein conjugates[76] has been documented. The phenomenon has been studied in depth by Maeda and coworkers who termed tumor accumulation of macromolecules the 'enhanced permeability and retention effect' (EPR),[76,77] attributing it to two factors; tumor vasculature often displaying a discontinuous endothelium which allows macromolecular extravasation to a greater extent than seen via most other endothelial barriers, and additionally lack of effective lymphatic drainage in tumors preventing clearance of the penetrant macromolecules and thus a tendency to accumulate within the tumor. This concept is described schematically in Figure 5 and the consensus view is now that the phenomenon is of central importance to the observed pharmacological activity of many macromolecular drug delivery systems. It remains to be shown whether passive accumulation of macromolecules by tumors is due primarily to endothelial leakage (inherent or

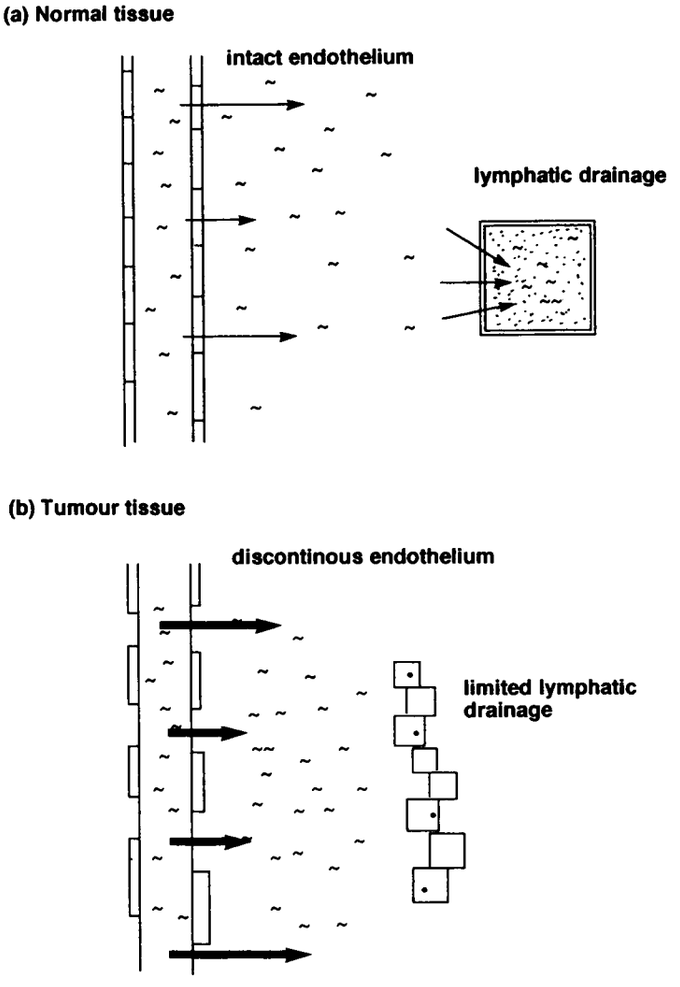


Figure 5. The EPR effect, after Maeda and Matsumura.[76] (a) Macromolecules have difficulty passing across the endothelial barrier of normal tissues and when they do escape into the extracellular fluid lymphatic drainage effectively removes them. (b) However, increased vascular permeability in tumor tissue and poor lymphatic drainage can lead to selective macromolecular retention in tumors.

induced by tumor secreted mediators), poor lymphatic drainage or enhanced pinocytic capture at the cellular level, but nonetheless the phenomenon is of undoubted significance in tumor localization of macromolecules in the animal models thus far studied, and has been extensively reviewed.[77–79]

Adsorptive pinocytosis facilitates more efficient substrate capture than fluid phase pinocytosis owing to membrane binding of the ligand—the conventional understanding of *active targeting*. Non-specific adsorptive pinocytosis usually occurs due to hydrophobic or charge-dependent interactions and is freqently independent of cell type. In contrast, receptor-mediated pinocytosis can be an highly efficient uptake process when cells display a high density of ligand-specific membrane receptors. Unfortunately many of the receptors identified in this category that have potential for tumor targeting (Table 3) often have a broad cellular distribution (e.g. transferrin, LDL, epidermal growth factor receptors) making them less than ideal targets. Exceptionally, receptors have been identified that appear cell-specific such as the galactose receptor of hepatocytes, which has been used to good effect for regional targeting.

There are many factors to consider when designing systems to target cell surface receptors; the homogeneity of receptor expression within a tumor, the number of receptors available per cell and their ligand affinity, the possibility of up–down regulation following exposure to the targeting ligand, and not least the cellular fate of the receptor–ligand complex. In particular a knowledge of the number of receptors expressed at any time is crucial as receptor saturation would obviously decrease efficiency of targeting (expressed as a percentage of the dose administered) if the dose given per bolus was increased without prior consideration of this point. However, it is worthy of acknowledgement that the benefit achieved by so called tumor-specific targeted systems has often been a simple pharmacokinetic advantage.

Use of hydrolytic and enzymatic degradation to control the rate of drug delivery

With knowledge that macromolecular conjugates accumulate in the extracellular fluid within a solid tumor mass, and are subsequently internalized by pinocytosis, there are opportunities to harness a number of natural biochemical mechanisms to control the rate of drug release. The acidic pH of some solid tumors, albeit an extracellular phenomenon, and the low pH within the endosomal and lysosomal compartments suggest that an acid-sensitive linker may provide both rate control of drug delivery and some tumor selectivity. Shen and Ryser[80] were the first to describe the synthesis of polymer (poly-D-lysine) conjugates containing pH-sensitive spacers; *N-cis*-aconityl and *N*-maleyl derivatives of daunomycin. When linked to Affi-Gel 701 (aminoethyl polyacrylamide beads) the *cis*-aconityl derivative had a half-life for hydrolysis of less than 3 h at pH 4, but it was much more stable at pH 6 with a half-life of more than 96 h (Figure 6).

Several proteases appear to have a fundamental role in metastasis.[81–83] These include type IV collagenase, transin/stromelysin and plasminogen activator active at neutral pH, and the lysosomal thiol-dependent proteases cathepsins B, L and lysosomal aspartic protease cathepsin D. Cathepsin D has a pH optimum in the acidic range (pH 2.8–5.0), whereas the thiol-dependent enzymes have much broader optima and retain considerable activity at neutral pH. Peptidyl spacers have been developed for attachment of anthracyclines to polymers[25,84–87] and proteins,[88] and their amino acid

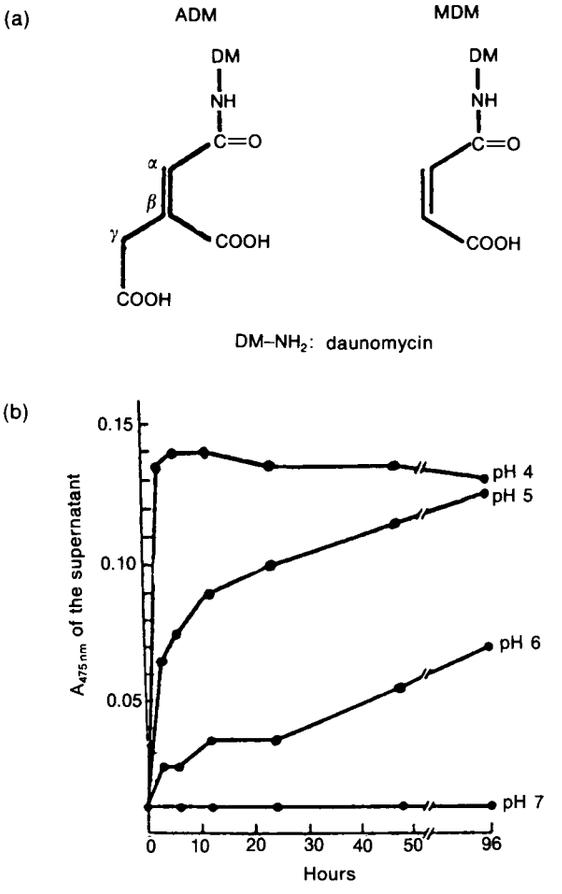


Figure 6. Formulae of *N-cis*-aconityl daunomycin and *N*-maleyl daunomycin (a) and pH sensitive release of daunomycin from ADM-AffiGel 701 (b). ADM-Affi-Gel 701 beads were suspended in 2.5 ml citrate phosphate buffer at pH 4, 5, 6 and 7 to give a final total concentration of 3×10^{-5} M of daunomycin. These gel suspensions were incubated at 37°C, centrifuged at various time intervals and the concentration of daunomycin in the supernatant was measured by the absorption at 475 nm (from Shen and Ryser,[80] with permission).

sequence can be specifically chosen to facilitate intracellular degradation by the lysosomal thiol-proteases. With increasing awareness that many metastatic tumors have increased levels of these lysosomal enzymes, use of macromolecular pro-drugs programmed for lysosomal degradation may be of wider potential than the originally perceived lysosomotropic approach.

HPMA copolymer conjugation

Over the last decade we (see Acknowledgements) have systematically developed HPMA copolymer

conjugates for the controlled release and targeting of antitumor agents. In this series of experiments we first tried to identify the fundamental polymer characteristics that would optimize performance as a drug carrier and subsequently used this knowledge to tailor-make specific conjugates that contained therapeutic agents [e.g. doxorubicin (Dox), daunomycin (Dnm), and melphalan (Mel)], already known to be clinically important in cancer chemotherapy. More recently, Kopecek *et al.*[89] have developed HPMA copolymers as targetable carriers for photoactivatable drugs.

HPMA is a biocompatible polymer originally developed in Czechoslovakia as a plasma expander[90] and known to be non-toxic at doses up to the maximum administrable of 30 g/kg. Conjugate synthesis has been described at length elsewhere,[91–97] but the method of preparation usually involves initial synthesis of a reactive polymeric precursor to which any compound containing an aliphatic amino group can subsequently be bound using an aminolysis reaction. This simple concept is so versatile that a variety of conjugates can be generated to contain different antitumor agents and targeting groups, whilst maintaining essentially the same molecular weight characteristics and therefore favorable biodistribution properties. Such aminolysis reactions have been carried out consecutively in organic or aqueous solutions allowing sequential binding of drugs, prodrugs, peptides or antibodies to the polymer carrier. Typical chemical structures are shown in Figures 7 and 8, and Table 4 lists some of the key studies undertaken with the HPMA copolymer system (although by no means a complete bibliography).

Design for controlled release

To facilitate their controlled release, antitumor agents have been covalently bound to HPMA copolymers via peptidyl spacers designed to limit

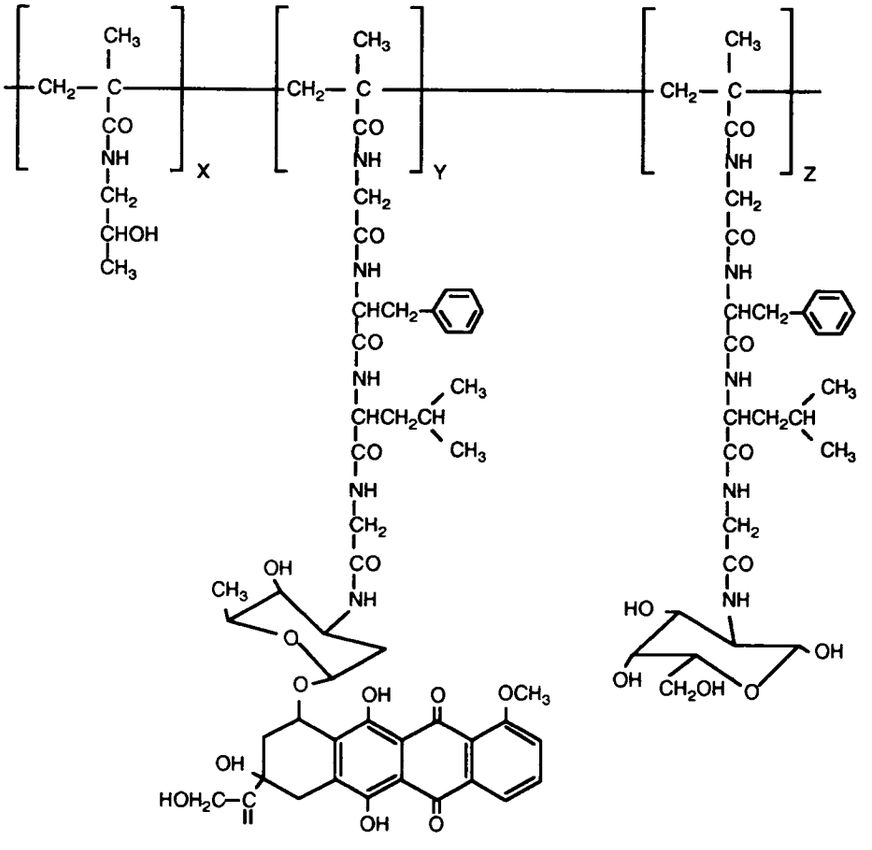


Figure 7. HPMA copolymer containing Dox and Gal bound via the peptidyl spacer Gly–Phe–Leu–Gly. HPMA (X), MA–Gly–Phe–Leu–Gly–Dox (Y) and MA–Gly–Phe–Leu–Gly–Gal (Z); the ratio of X:Y:Z is 94:2:4.

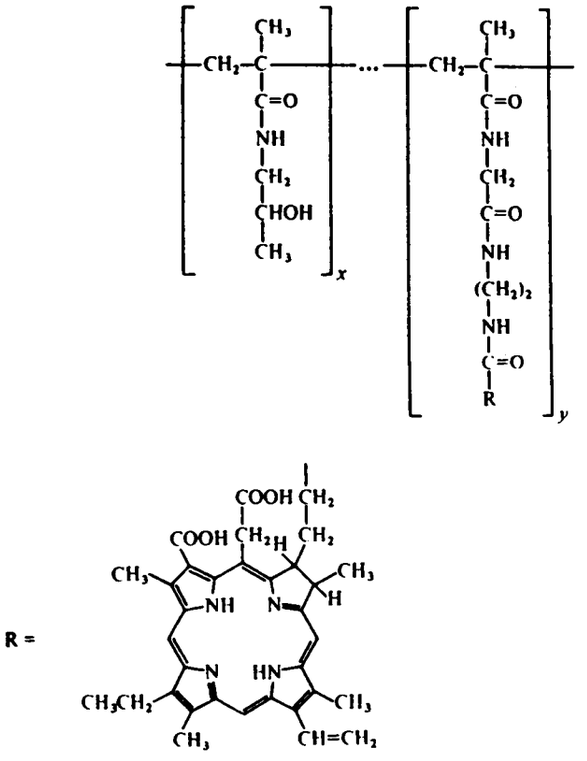


Figure 8. HPMA copolymer conjugate containing chlorin e_6 (3.6 mol%) (from Krinick *et al.*,[128] with permission).

drug release in plasma and serum[98] [using the model drug *p*-nitroaniline (NAp) serum degradation was shown to be less than 5% in 5 h (Table 5)], but be amenable to degradation by the lysosomal proteases,[81,84,99] particularly cathepsins B, H and L.[86,100] In the early studies mixtures of isolated rat liver lysosomal enzymes (tritosomes) were used as the most convenient *in vitro* test system to study spacer degradation. Later, purified bovine spleen enzymes, cathepsins B, H and C were also used in such assays. Relevance to the human situation may be questioned, but it has recently been confirmed that human liver cathepsin B has equivalent ability to degrade peptidyl spacers in HPMA conjugates to liberate Dox.[130] Length and composition of the amino acid spacer was shown *in vitro* to influence the rate of release of Mel and Dox over a broad range, with between below 10% released per day to more than 80% per day.[26,101] The extent of Dox release from conjugates containing tri- and tetrapeptidyl spacers when incubated with tritosomes is shown in Figure 9. Non-biodegradable conjugates do not show antitumor activity *in vivo* in any model system, confirming the need for drug release to mediate pharmacological activity. A correlation has been observed between the rate of Dox[101] and Mel[26] release and antitumor activity *in vivo*. Those conjugates releasing Mel more quickly showed greater activity against Walker sarcoma,[26] but interestingly, polymer–Dox conjugates containing the slower releasing tripeptide spacers Gly–Phe–Gly and Gly–Leu–Gly administered intraperitoneally (dosing schedule days 1, 2 and 3 each 5 mg/kg) to treat an intraperitoneal L1210 ascitic tumor proved more effective than conjugates containing the faster releasing tetrapeptide spacers.[101] In this case the slower release kinetics which maintained a sustained, but low level of Dox, were obviously advantageous. A substantial library of potential peptidyl spacers has now been described such that conjugates can be synthesized to give the optimal release profile for each pendant drug and the stage of the particular tumor to be treated.

Pharmacokinetics and targeting

Characteristics that determine biodistribution *in vivo* have been reported. HPMA copolymer preparations containing a small amount of tyrosinamide (about 1 mol%) (to permit radioiodination) and with of narrow polydispersity (about 1.2) were prepared in the range M_W = 12 000–778 000 and their body distribution monitored after intravenous, intraperitoneal or subcutaneous administration to rats.[102] The effect of molecular weight on plasma clearance is shown in Figure 10, and as would be predicted those polymers whose molecular weight is lower than the renal threshold are cleared very rapidly, whereas high molecular weight polymers remain in the circulation for long periods, being retained in the body and showing greater accumulation in the reticuloendothelial system.[102] Goddard *et al.*[103] found similarly that an HPMA copolymer containing tyramine (4 mol%) also showed a size-dependent body distribution, and they suggested that lower molecular weight fractions of this polymer could be found in skin and muscle after 48 h. In all cases HPMA copolymers were not subject to the very rapid clearance by the reticuloendothelial system observed with most liposomes and particulate carriers, and these polymers are seemingly taken into cells indiscriminately by the mechanism of fluid phase pinocytosis.

As a result of these studies, HPMA copolymers of M_W ~20 000 were chosen as optimum for development as a clinical drug delivery system. Conjugates of this molecular weight are relatively

Table 4. Studies carried out using HPMA copolymers during their development as drug carriers for use in cancer chemotherapy

Study	Author	Reference
Design of peptidyl spacers for controlled degradation		
Mixtures of lysosomal enzymes	Duncan *et al.*	84, 85, 99
Cathepsin B	Rejmanova *et al.*	86
Cathepsins B, H and L	Subr *et al.*	98
Doxorubicin release	Subr *et al.*	101
Melphalan release	Duncan *et al.*	26
Factors governing body distribution		
Effect of M_w and route of administration	Seymour *et al.*	102
	Goddard *et al.*	103
	Cartlidge *et al.*	104
Effect of pendent sugar residues including galactose body distribution	Seymour *et al.*	105
	Duncan *et al.*	106
Targeting using galactose	Chytry *et al.*	107
Targeting using MSH	O'Hare *et al.*	108
Transferrin–polymer conjugates	Flanagan *et al.*	109
B72.3–polymer conjugates	Seymour *et al.*	110
Pharmacokinetics of polymer–doxorubicin	Seymour *et al.*	111
Pharmacokinetics of polymer–gal-doxorubicin	Seymour *et al.*	112
Body distribution of polymer–daunomycin	Rihova *et al.*	113
Distribution of polymer–daunomycin into a solid tumor	Cassidy *et al.*	114
Distribution of polymer–doxorubicin into a solid tumor	Wedge *et al.*	115
	Seymour *et al.*	116
Factors effecting cell uptake/subcellular distribution		
Effect of M_w on pinocytic uptake	Cartlidge *et al.*	117
Receptor mediated interaction of polymer–galactose with hepatoma	O'Hare *et al.*	118
Subcellular distribution of HPMA copolymers	Duncan *et al.*	106
	McCormick *et al.*	119
	Flanagan *et al.*	120
	Wedge *et al.*	121
Biocompatibility		
Immunogenicity of HPMA conjugates	Rihova *et al.*	122
	Vetvicka *et al.*	123
Immunogenicity and bone marrow toxicity of polymer doxorubicin	Flanagan *et al.*	124
	Rihova *et al.*	96
Interaction with complement	Simeckova *et al.*	125
Cardiotoxicity of polymer–doxorubicin	Yeung *et al.*	126
Conjugates containing antitumor agents[a]		
Daunomycin (and galactose)	Duncan *et al.*	25
Doxorubicin (and galactose)	Duncan *et al.*	24, 56
Melphalan (and sugar residues)	Duncan *et al.*	26
	Ulbrich *et al.*	127
Chlorin e_6 (and antibodies)	Krinick *et al.*	128
Bis(2-chloroethyl)amine	Ringsdorf *et al.*	129
Doxorubicin (and MSH)	O'Hare *et al.*	108

[a] Additional targeting groups were used in some studies.

small, e.g. polymer–Dox has a diameter of about 8 nm measured by light scattering[131] and thus should have maximum opportunity for both tumor penetration, and effective excretion from the body. Studies on the biodistribution of polymer–Dox[111] and polymer–Mel[26] show clearly that macromolecular conjugation changes drug pharmacokinetics dramatically. The plasma half-life increases from below 5 min to 1 h for free and bound Dox, respectively, and from 90 min to 5 h in the case of Mel (see Figures 11 and 12). It is noteworthy that the stability of the peptidyl linker in the circulation ensures that no significant amounts of free Dox are detected in the bloodstream after administration of polymer–Dox (Figure 11). The prolonged plasma half-life of the conjugate is probably instrumental

Table 5. Degradation of HPMA copolymer side chains by rat plasma and serum (from Rejmanova *et al.*[98])

Copolymer side chain[a]	Percentage *p*-nitroaniline released/5 h	
	plasma	serum
P–Gly–Leu–Ala–NAp	0.9	0.9
P–Gly–Phe–Ala–NAp	0.0	0.9
P–Ala–Gly–Val–Phe–NAp	0.0	0.5
P–Gly–Phe–Phe–Ala–NAp	1.0	2.2
P–Gly–Phe–Phe–Leu–NAp	1.5	2.1
P–Gly–Phe–Leu–Gly–NAp	1.5	1.7
P–Gly–Phe–Tyr–Ala–NAp	1.3	1.3
P–Gly–Phe–Leu–Gly–Phe–NAp	3.3	3.8
P–Gly–Gly–Phe–Leu–Gly–Phe–NAp	3.5	5.1

[a] P = HPMA copolymer backbone; NAp = *p*-nitroanilide.

in producing the EPR now observed using a number of model tumors including Walker sarcoma,[114] B16 melanoma[132] and sarcoma-180.[116] Cassidy *et al.*[114] were the first to demonstrate selective tumor accumulation of HPMA copolymer conjugates (Figure 13). They compared tumor levels of Dnm following intravenous administration of free and conjugated drug (5 mg/kg Dnm

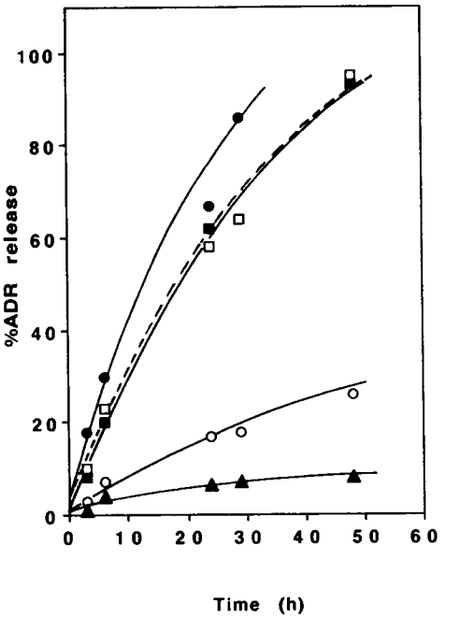


Figure 9. Time course of enzymatic hydrolysis of HPMA polymeric prodrugs containing doxorubicin (ADR) by tritosomes (○) Gly–Leu–Gly; (▲) Gly–Phe–Gly; (□) Gly–Phe–Leu–Gly; (■) Gly–Phe–Leu–Gly (polymer also containing galactosamine); (●) Gly–Leu–Phe–Gly (from Subr *et al.*[101]).

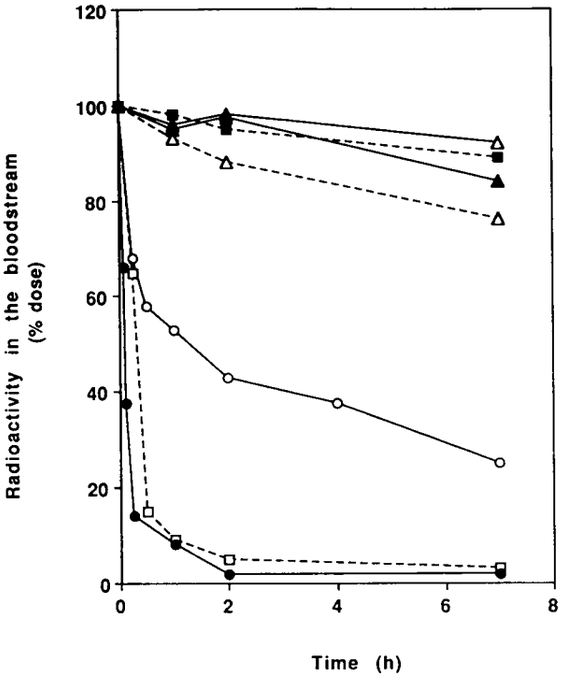


Figure 10. Effect of molecular weight on the blood clearance of ^{125}I-labeled HPMA copolymers following intravenous administration to rats. Radioactivity in the bloodstream (% dose) is shown for HPMA copolymers of M_w 778 000 (△—△); 556 000 (■---■); 148 000 (▲—▲); 78 000 (△---△); 40 000 (○—○); 22 000 (□—□); 12 000 (●—●). The mean ± SE of at least three determinations is known (from Seymour *et al.*[102]).

equivalent) in rats bearing subcutaneous Walker sarcoma and found a 4-fold increase in area under curve (AUC) in the tumor when Dnm was administered in conjugate form (it is now known that a substantial amount of drug was still awaiting release in the tumor at 24 h[24]). The tumor accumulation of HPMA copolymer–Dox (up to 20% dose/g) measured recently using subcutaneous sarcoma-180 model was considerably greater than reported previously for proteins (8% dose/g) measured under identical conditions.[116] Preliminary experiments using polymers of discreet sizes showed no size dependence of polymer uptake into tumors up to a molecular weight of 556 000,[116] but this observation awaits confirmation.

Incorporation of targeting residues into HPMA copolymer conjugates (Table 4) has been used to facilitate first- and second-order targeting. HPMA copolymer–Dox conjugates containing additionally galactosamine (Gal) are avidly captured by the liver due to interaction with the hepatocyte galactose-recognizing receptor[56] which physiologically serves

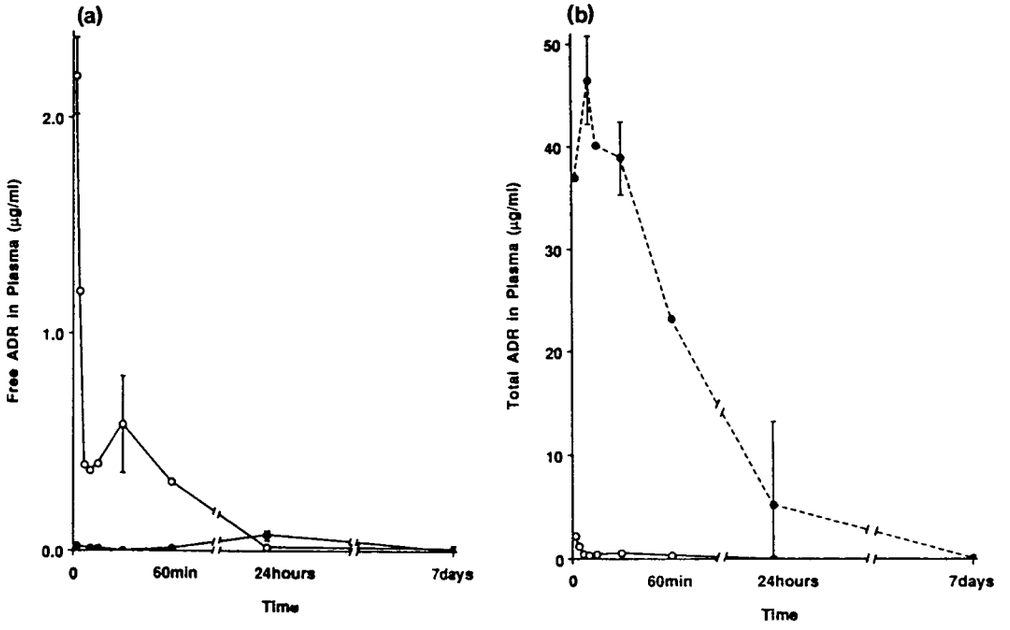


Figure 11. Plasma clearance of doxorubicin (ADR) and polymer-bound ADR. Panel (a) shows the levels of freely-extractable ADR following the administration of (○) free ADR and (●) polymer-bound ADR. Panel (b) shows the total drug-levels observed following administration of (○) free ADR and (●) polymer-bound ADR (from Seymour *et al.*[111]).

to remove asialoglycoproteins from the circulation.[133] When administered intravenously at low dose (equivalent to a Dox dose of 0.5 mg/kg) polymer–Dox–Gal is captured very effectively by the liver (more than 80% of the dose administered); however, receptor saturation does occur at higher doses, and this significantly decreases the desired specificity[112] (Figure 14). Therefore, an ideal clinical protocol would require either repeated low bolus dose or continuous infusion. HPMA copolymer–Dox–Gal was designed to facilitate first-order targeting to the liver for treatment of primary and secondary liver disease. However, usefulness of first-order targeting antitumor agents to the liver has been hotly debated as most drug will be delivered to normal liver, with hepatotoxicity or inadequate redistribution of drug to tumor as a potential consequence. Preliminary studies following intravenous administration of HPMA–Dnm–Gal (10 mg/kg) showed no change in the plasma levels of transaminases and alkaline phosphatase indicating no hepatotoxicity.[134] Although human hepatoma does express the galactose receptor, and it has been shown that the human lines $HepG_2$ and Alexander are recognized by HPMA copolymer–Gal,[118] it is known that the number of galactose receptors present on the surface of tumor cells is often reduced, or may even be absent in some patients,[135] and therefore it remains conjecture whether first-order targeting to the liver will be clinically useful or not.

It has recently been shown that intravenous administration of HPMA copolymer–Dnm–Gal leads to a greater initial percentage of lysosomally associated drug (5-fold greater, measured using subcellular fractionation) in the liver compared with an equivalent dose of Dnm. This is followed by a continual transfer of the liberated drug to the cytoplasmic fraction resulting in a sustained high cellular and nuclear Dnm concentration over 48 h.[121] These data demonstrate in a model system the ability of a macromolecular prodrug to increase and maintain the intracellular drug concentration within the target tissue.

Although HPMA copolymer conjugates display effective first-order targeting to the liver, and advantageous pharmacokinetics with ability to concentrate passively in solid tumors, the feasibility of tumor-specific second-order targeting is continually being pursued. A large number of HPMA copolymer–polyclonal and monoclonal antibody conjugates have been described, including: anti-Thy-1.2, anti-Ia^k, B72.3, B3/25 (specific for the transferrin receptor) antibodies. Rihova *et al.*[136,137] initiated this programme with the particular aim of targeting immunosuppressive drugs *in vivo*, hence

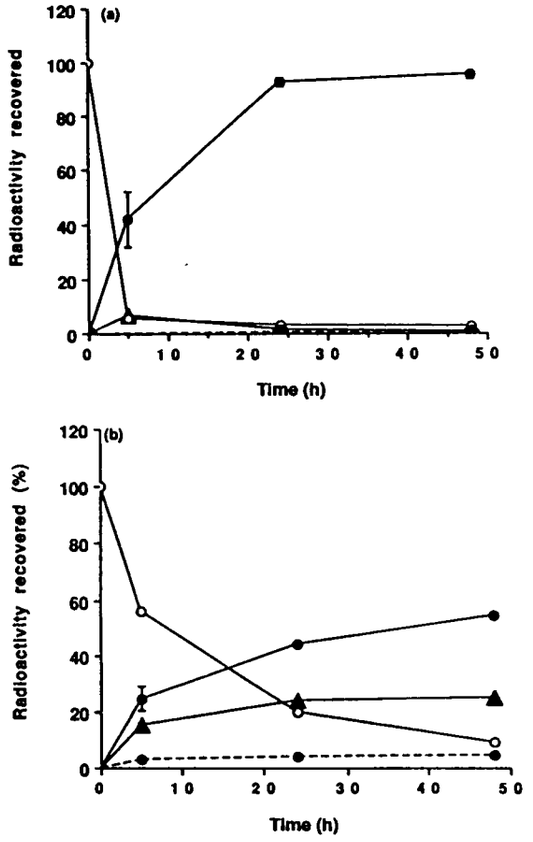


Figure 12. Body distribution of [^{3}H]Mel (ME) and ^{125}I-labeled P–Gly–Phe–Gly–Gly–ME after intravenous administration to tumor bearing rats. The body distribution of radioactivity is shown following administration of [^{3}H]Mel (a) and ^{125}I-labeled polymer–Mel (b). Key: blood (○—○); urine and faeces (●—●); liver (▲—▲); tumor (●---●) (from Duncan *et al.*[26]).

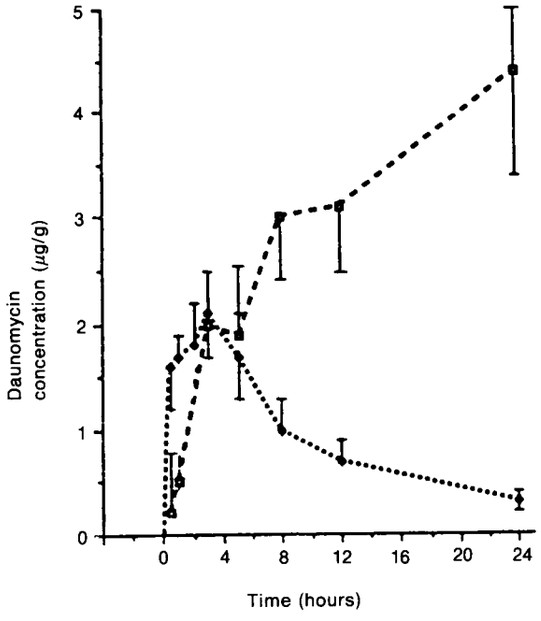


Figure 13. Free Dnm of HPMA copolymer Dnm (P–Gly–Phe–Leu–Gly–Dnm) was administered intravenously to Wistar rats bearing subcutaneous Walker 256 tumor at a Dnm equivalent dose of 5 mg/kg. Free Dnm levels were measured by HPLC after administration of free drug (●---●) or (□---□).

the choice of antibodies which recognize lymphocyte-specific antigens. Two approaches have been described for binding antibodies to the polymeric carrier. Most studies have used conjugates prepared using the above-mentioned aminolysis reaction which can theoretically be optimized to produce 1:1 conjugation of antibody to polymer. However, in practice this is a poorly controlled reaction which leads to multiple products including polymers linked to antibody via the hypervariable region with resultant inactivation. Although HPMA copolymer–anti-Thy-1.2 conjugates prepared using this method have been reported to retain 20–80% of their antigen recognizing activity,[136] similar conjugates containing B72.3 did not localize specifically in the xenograft tumor LS174T, although parent antibody localized up to 25%/g.[110] Recently, Krinick *et al.*[128] have reported improved biological activity of chlorin e_6–HPMA conjugates containing anti-Thy-1.2 antibody bound via oxidized carbohydrate in the antibody Fc region. This method of conjugation affords some hope to retain the specificity of polymer conjugates, but if polymer conjugates containing antibody or antibody fragments are to be successful considerable development is still needed to optimize the chemistry of attachment. This series of experiments does however highlight the advantages of using HPMA copolymer–protein conjugates. It has been repeatedly demonstrated that covalent conjugation of antibodies and proteins to HPMA copolymers reduces their immunogenicity, measured as a decreased IgG response.[124,138] Conjugation can also be used to modify protein pharmacokinetics, e.g. the plasma half-life of Fab' derived from B72.3 was extended 10-fold (from 35 min to 6 h) when polymer-bound.[110]

Antitumor activity

Relatively potent antitumor agents already in clinical use were selected for the synthesis of the

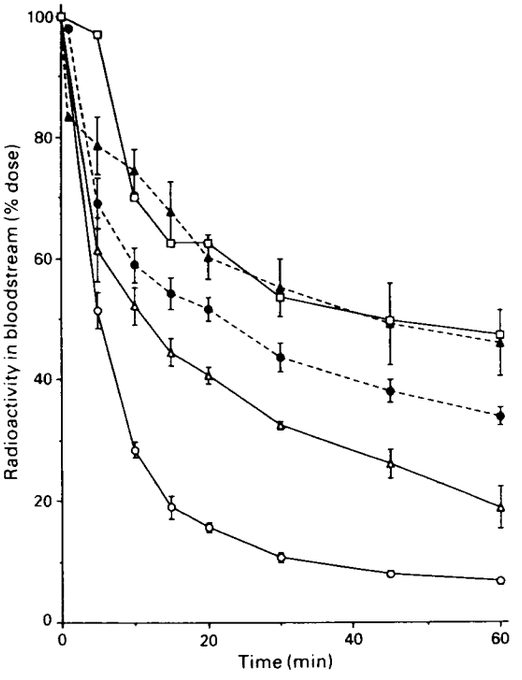


Figure 14. Effect of administered dose on the blood clearance of HPMA copolymer–Dox containing Gal. Blood clearance was measured following intravenous bolus administration of 0.05 mg/kg (○—○), 0.5 mg/kg (△—△), 5 mg/kg (●---●) or 15.0 mg/kg (▲---▲) related to the Dox content of the conjugate. Blood clearance of HPMA copolymer–Dox without Gal (□—□) administered at a dose of 0.05 mg Dox/kg is shown for comparison. Each point represents the mean of at least three determinations ±SE (from Seymour *et al.*[112]).

first HPMA conjugates. In particular the anthracycline antibiotics Dox and Dnm (Dox being the antitumor agent showing the widest spectrum of antitumor activity), and alkylating agents such as Melphalan [(Mel) and sarcolysin (Sle), the D,L form, Mel being the L isomer] and bis(2-chloroethyl) amine.

Anthracycline conjugates. Early studies confirmed that polymers covalently bound to Dox and Dnm via biodegradable spacers showed activity against the mouse leukemic model L1210 (intraperitoneal). Conjugates with non-degradable spacers were not active.[25,56] Most emphasis has recently focused on development of Dox conjugates for clinical evaluation and two HPMA copolymer–Dox conjugates are progressing to a phase I/II trial. Both have a Dox loading of approximately 7 wt% (2 mol%), M_w ~20 000 and narrow polydispersity (M_w/M_n <1.4), and are some 10 times more soluble than free Dox and the second polymer contains additionally galactosamine (4 mol%). When administered intraperitoneally to treat L1210 as an ascitic tumor model, both conjugates displayed a considerably higher T/C than free Dox, and gave rise to long-term survivors. A total cumulative dose of 90 mg/kg of conjugate given intraperitoneally produced no marked toxicity (Table 6). Polymer–Dox administered intravenously was less active against intraperitoneal L1210, but comparisons are difficult as a single, higher bolus dose was given in these studies; with a single intravenous dose the LD_{50} was 100 mg/kg. L1210 leukemia is known to be particularly sensitive to anthracycline therapy so HPMA–Dox conjugates were also evaluated against a panel of solid tumor models including M5076 (Table 7), an established subcutaneous P388 model (Figure 16), Walker sarcoma[114] and an established B16 melanoma model.[108] Antitumor activity was observed in all cases, but perhaps the most encouraging was that seen when using the human colon xenograft LS174T.[24,139] The M5076 model responded well to conjugate treatment with appearance of long-term survivors where previously free Dox produced none. However, the dosing schedule used was such that first drug administration occurred before the M5076 tumors were of palpable size (Table 7). Subcutaneous tumors allowed to develop to palpable size prior to any treatment showed either marked regression (Figure 16) following intraperitoneal or intravenous injection of the polymer–Dox conjugate, in the case of P388 and Walker sarcoma,[24] or control of tumor progression in the case of B16 melanoma[108] and the xenograft LS174T.[139] In all cases, the responses seen using conjugates were far superior to those observed for free Dox.

To investigate the therapeutic benefit of liver targeting with galactose, efficacy of HPMA copolymer–Dox–Gal was monitored using two liver metastatic model systems; intravenous M5076[24] and B16 melanoma.[140] Data obtained using the M5076 model are shown in Table 8, and two interesting factors emerge. First, although both polymer–Dox and the Gal-containing equivalent gave rise to long-term survivors, and an increased T/C, surprisingly the untargeted conjugate did better. Second, it would appear that the Gal-containing derivative had decreased activity as the dose increased. The latter could be easily attributed to receptor saturation, but one would then predict conjugate behavior corresponding to that of the non-targeted form. The results are somewhat baffling unless the differences in performance relate

Table 6. Treatment of mice bearing L1210 leukaemia (intraperitoneally) with HPMA copolymer–Dox (from Duncan *et al.*[24])

Treatment	Dose (mg/kg)[a]	T/C[b] (%)	Toxic deaths	Long-term survivors[c]
Intraperitoneal administration (three doses, days 1, 2 and 3)				
Dox	2.5	168	0/30	1/30
	5.0	214	3/40	0/40
	10.0	80	40/40	0/40
Polymer–Dox	2.5	113	0/10	0/10
	5.0	138	0/10	0/10
	10.0	170	0/30	1/30
	20.0	231	0/10	0/10
	30.0	>430	2/30	8/30
	50.0	110	20/20	0/20
Polymer–Dox–Gal	2.5	113	0/10	0/10
	5.0	138	0/10	0/10
	10.0	203	0/20	2/20
	20.0	256	0/10	2/10
	30.0	>762	0/20	17/20
	50.0	113	10/10	0/10
Intravenous administration (single dose)				
Dox	13	167	0/10	0/10
	16.9	167	0/10	0/10
	22	183	0/10	0/10
Polymer–Dox	25.0	125	0/10	0/10
	50	150	0/10	0/10
	75	183	0/10	0/10
	100	233	5/10	0/10

[a] Represents the equivalent Dox dose.
[b] Ratio of median survival of the test group (T) to that of untreated control (C) expressed as a percentage.
[c] Animals surviving until 60 days.

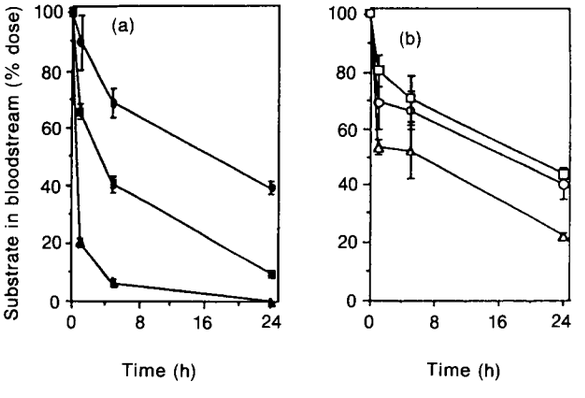


Figure 15. Blood clearance of B72.3 antibody, its fragments and HPMA copolymer conjugates. Substrates were injected intravenously (400 μg), and blood samples taken over the subsequent 24 h and blood clearance is shown. (a) Unmodified proteins, ●: intact IgG, ▲: Fab' fragment, ■: Fab'_2 fragment. (b) HPMA copolymer conjugates, ○: HPMA–IgG, △: HPMA–Fab', □: HPMA–Fab'_2 (from Seymour *et al.*[110]).

to drug distribution within normal liver and tumor. Similarly conjugates were found to be equi-effective when using a liver metastatic B16 melanoma model in combination with fractionated doses of polymer –Dox with and without galactose.[140] Analysis of pharmacokinetics and efficacy with aid of liver metastatic models is still underway.

For tumor-specific localization it appears that antibody fragments, smaller proteins or peptides would afford greatest opportunity to achieve second-order targeting of HPMA–drug conjugates. The transferrin-containing conjugates synthesized to utilize the concept that tumor cells have a higher density of transferrin receptors showed reasonable interaction with the transferrin receptor *in vitro*, but when efficacy of Dnm conjugates containing transferrin was evaluated against L1210 *in vivo* their activity was less than seen for the non-targeted equivalent (Table 9), probably due to the widespread distribution of the transferrin receptor in the

Table 7. Treatment of mice bearing M5076 (subcutaneously[a]) with Dox or HPMA copolymer–Dox (from Duncan *et al.*[24])

Treatment	Dose[b] (mg/kg)	T/C[c] (%)	Toxic deaths	Long-term survivors[d]
Intravenous administration (three doses, days 5, 9 and 15)				
None				0/10
Dox	2.5	114	0/10	0/10
	5	124	0/10	0/10
	10	125	2/10	0/10
Polymer–Dox	5	163	0/10	0/10
	10	220	0/10	1/10
	20	319	0/10	4/10
Polymer–Dox–Gal	5	128	0/10	0/10
	10	146	0/10	0/10
	20	178	0/10	0/10
Intraperitoneal administration (three doses, days 5, 9 and 15)				
Dox	5	78	0/10	0/10
	10	102	4/10	0/10
	20	24	10/10	0/10
Polymer–Dox	5	157	0/9	0/9
	10	174	0/10	1/10
	20	>264	0/10	6/10
Polymer–Dox–Gal	5	123	0/10	0/10
	10	155	0/10	0/10
	20	167	0/10	2/10

[a] 5×10^5 tumor cells were administered subcutaneously on day 0.
[b] Represents the equivalent Dox dose.
[c] Ratio of median survival of the test group (T) to that of untreated control (C) expressed as a percentage.
[d] Animals surviving until 120 days.

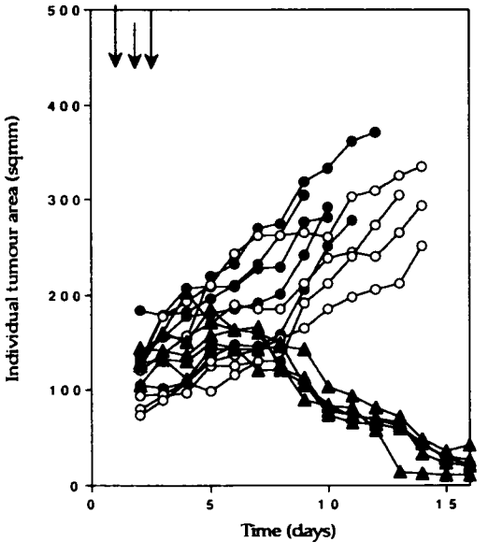


Figure 16. Effect of intraperitoneal administration of (○) Dox (3 × 3 mg/kg) and (▲) HPMA copolymer–Dox (3 × 18 mg/kg relative to Dox) on tumor area of an established subcutaneous P388 tumor. Each line represented data obtained with one animal, the dosing schedule is shown and also data obtained with phosphate buffered saline treated animals (●) (from Wedge[115]).

body and hence lack of selectivity, and additionally limited ability of the transferrin conjugate to reach to lysosomal compartment and thus liberate drug intracellularly.[109] In contrast, conjugates containing melanocyte stimulating hormone (MSH) display considerable promise for targeting drugs to malignant melanoma. Like MSH, MSH-containing conjugates have the ability to stimulate tyrosinase activity in melanoma cells *in vitro*,[108] showed increased pinocytic uptake by melanoma cells *in vitro* and HPMA copolymer–Dox–MSH conjugates had greater activity against subcutaneous B16 melanoma[108] and a B16 melanoma metastatic liver model[140] when compared with equivalent conjugates without MSH.

Alkylating agents. In vivo investigation of HPMA copolymer conjugates containing Sle or Mel has been limited to evaluation against subcutaneous Walker sarcoma[26] and B16 melanoma.[141] Administration of polymer–Sle conjugates intraperitoneally to rats on day 1 after tumor inoculation (10^6 cells, subcutaneous) either prevented tumor establishment or reduced tumor size measured on day

Table 8. Treatment of mice bearing intravenously injected M5076[a] with Dox or HPMA copolymer–Dox (from Duncan *et al.*[24])

Treatment	Dose[b] (mg/kg)	Liver weight[c] (mg)	T/C (%)	Toxic deaths	Long-term survivors
Controls		1552			0/10
Dox	5	1160	133	0/10	0/10
	10	1013	148	0/10	1/10
P–Gly–Phe–Leu–Gly–Dox	5	969	159	0/10	1/10
	10	970	175	0/10	1/10
	20	1122	234	0/10	4/10
	30	1122	>244	0/10	8/10
P(Gly–Phe–Leu–Gly–Dox)(Gly–Phe–Leu–Gly–Gal)	5	915	184	0/10	3/10
	10	1073	136	0/10	1/10
	20	1115	170	0/10	0/10

[a] 10^5 tumor cells were injected intravenously on day 0.
[b] Treatment was given intravenously on days 3, 7 and 11. Dose represents Dox equivalent.
[c] Liver weight gives an indication of metastatic disease.

15. An exception being the non-degradable conjugate containing Gly–Sle side chains. The extent of antitumor activity was shown to correlate with the rate of enzymatic drug release from conjugate measured *in vitro*. Treatment (intraperitoneally on day 1) of B16 melanoma (10^6 cells administered intraperitoneally) with Mel or polymer–Gly–Phe–Leu–Gly–Mel at doses of 20, 10 and 5 mg/kg with respect to Mel confirmed that drug conjugation reduced toxicity markedly, but unfortunately also reduced efficacy. Mel was very toxic at 20 mg/kg, but a dose of 10 mg/kg produced two out of five long-term survivors and a T/C = 188. Polymer–Mel at 20 mg/kg was non-toxic, but the T/C was only 133 with no long-term survivors. It was noteworthy that lower dose Mel, both free and polymer-bound, was more effective in this assay so dose escalation with the polymer form may be of limited benefit in terms of increased efficacy.

Photoactivatable drugs. A relatively new departure has been extension of the HPMA copolymer approach for targeted delivery of photoactivatable compounds.[89] Activation of drug *in situ* after tumor localization would obviously provide an additional opportunity to ensure selectivity. Although *in vivo* studies have not yet been reported, *in vitro* analysis of conjugates containing either galactosamine, for targeting to hepatoma (Alexander cells were

Table 9. Treatment of DBA_2 mice bearing L1210 leukemia[a] with Dnm and HPMA copolymer–Dnm conjugates (from Flanagan *et al.*[109])

Treatment	Dose (mg/kg)	Day of removal	Mean survival (±SE)	Long-term survivors
None		18, 18, 22, 23, 31	22.4 ± 2.4	none
Transferrin (Tf)	44.3	21, 24, 25, 29, 29	26.6 ± 1.5	none
Dnm	10	9, 9, 9, 9, 9	9.0 ± 0**	none
P–Gly–Gly–Tf	44.3 (Tf)	18, 18, 19, 19, 31	21.0 ± 2.5^{NS}	none
P(Gly–Gly–Tf)(Gly–Gly–Dnm)	44.3 (Tf) 10.0 (Dnm)	18, 21, 21, 24, 26	22.0 ± 1.4^{NS}	none
P–Gly–Phe–Leu–Gly–Dnm	10.0	24, 31, 39	31.4 ± 4.3*	2/5
P(Gly–Phe–Leu–Gly–Tf)(Gly–Phe–Leu–Gly–Dnm)	44.3 (Tf) 10.0 (Dnm)	23, 26, 28, 31, 33	28.2 ± 1.8^{NS}	none

*$p = 0.05$; **$p = 0.001$; ^{NS}non-significant.
[a]Mice were inoculated intraperitoneally with L1210 leukemia and treated intraperitoneally with either drug or HPMA copolymer–drug conjugates.

used[142]) or anti-Thy-1.2 antibodies with mouse splenocytes from A/J mice as their target,[89] showed that the targeted conjugates, when subjected to light activation, were more biologically active than the non-targeted ones.

Mechanism of action of HPMA–copolymer drug conjugates

Although the precise mechanism of conjugate action is still unclear, it is known that controlled release of drug is essential for activity. In addition, passive and active targeting and the associated decrease in peripheral toxicity all contribute greatly to the activities seen. There is also a possibility that a drug conjugation may potentiate an antitumor immune response. HPMA copolymer conjugates containing Dox are much less toxic than free drug. They have an LD_{50} that is 5- to 10-fold higher than free Dox (Table 6). Furthermore, Rihova *et al.*[96] have shown that Dox conjugation leads to a reduction in bone marrow toxicity (Table 10) and have confirmed that HPMA copolymer–Dox conjugates do not elicit a significant antibody response (IgG or IgM) when administered repeatedly to two inbred (A/J and C57BL/10ScSn) strains of mice (Table 11). The small IgG response observed after intraperitoneal administration of conjugate is not sufficient to alter the observed antitumor activity seen against L1210 *in vivo*—confirmed by preimmunizing animals and then inoculating and treating the developing tumor.[143]

The major dose-limiting toxicity associated with clinical use of anthracyclines is cardiotoxicity. Pharmacokinetic studies show that administration of polymer conjugates containing either Dnm[114] or Dox[111] decrease up to 100-fold the level of anthracycline measured (by HPLC) in the heart when compared with equi-dose of free drug. Using a sophisticated system to monitor cardiac function in rats, Yeung *et al.*[126] verified that these pharmacokinetic measurements translate into a physiological response. Administration of several different HPMA copolymer–Dox conjugates to rats at a Dox dose equivalent to 4 mg/kg caused no significant change in cardiac output, whereas this dose of free drug decreased cardiac output and indeed all animals died within 12 weeks (Figure 17). A dose escalation study with polymer conjugates is underway.

The possibility that drug conjugation may lead to immunostimulatory activity is an interesting one, as it is generally agreed that Dox and Mel display immunostimulatory activity, particularly at low dose.[144,145] It was clearly shown that treatment of Walker sarcoma with polymer–Mel led to an increase in the numbers of macrophages, and B and T lymphocytes recovered in tumor tissue when compared with identical tumors treated weith Mel.[26] Results obtained when using the same model, but treatment with polymer–Dox were, however, not conclusive.[146,147] It is theoretically possible that the slow, sustained release of drug from the polymeric carrier mimics the situation seen during low-dose chemotherapy, but other explanations are plausible

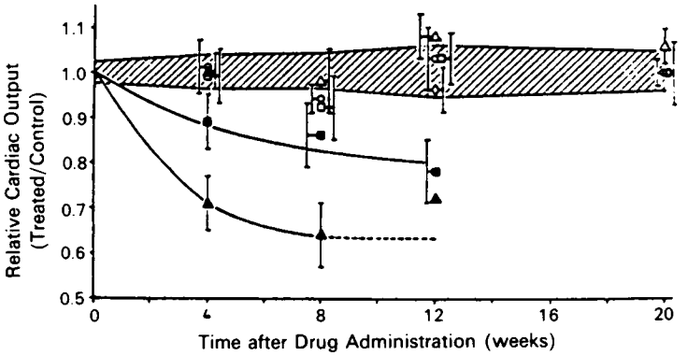


Figure 17. Time related changes in the relative cardiac output of rats (±SE) after single intravenous doses of Dox or HPMA copolymer–Dox (□) P-Gly-Gly-Dox; (○) P-Gly-Phe-Leu-Gly-Dox; (△) P-Gly-Phe-Leu-Gly-Dox-Gal; (◇) copolymer HPMA copolymer; (■) mixture of HPMA copolymer + free Dox; (▲) free Dox. The hatched area represents the SE for age-matched control animals (from Yeung *et al.*,[126] with permission).

Table 10. Spleen colony-forming units (CFUs) detected in irradiated recipient mice after injection of bone marrow harvested from mice injected with free Dox or HPMA copolymers containing Dox (from Rihova *et al.*,[96] with permission)

Sample	Immunization protocol[a]	Route of application	Number of CFUs per spleen detected on day	
			3	6
P–Gly–Phe–Leu–Gly–Dox	A	intravenously	—	26 ± 4
		subcutaneously	—	29 ± 3
		orally	—	30 ± 4
	B	intravenously	26 ± 5	27 ± 4
		subcutaneously	25 ± 3	28 ± 4
		orally	27 ± 2	27 ± 5
P(Gly–Phe–Leu–Gly–Dox)(Gly–Phe–Leu–Gly–Gal)	A	intravenously	—	30 ± 3
		subcutaneously	—	26 ± 5
		orally	—	30 ± 5
	B	intravenously	23 ± 3	29 ± 5
		subcutaneously	26 ± 1	27 ± 3
		orally	27 ± 4	27 ± 3
Dox	A	intravenously	—	14 ± 2
		subcutaneously	—	18 ± 3
		orally	—	28 ± 5
	B	intravenously	5 ± 1	13 ± 3
		subcutaneously	11 ± 3	19 ± 3
		orally	25 ± 5	27 ± 5
None (control)	—	—	—	25 ± 4

[a] A, immunization daily five times with 300 μg of polymer (22–25 μg of Dox per immunization). On the sixth day after the last treatment cells from bone marrow were isolated. B, Immunization every third day, five times with 300 μg of polymer (22–25 μg of Dox immunization). On the third and sixth days after the last treatment cells from bone marrow isolated. Data are expressed as the average of the triplicate ± SE.

Table 11. Immunogenicity of HPMA copolymer–Dox samples following immunization every third day (from Rihova *et al*,[96] with permission)

Sample	Dose (μg)		Route of application	Antibody titre			
	copolymer	Dox		A/J mice		C57BL/10ScSn mice	
				3rd day	6th day	3rd day	6th day
P–Gly–Phe–Leu–Gly–Dox	300	25.5	intravenously	1/64	1/128	1/32	1/128
			subcutaneously	1/64	1/64	1/128	1/128
			orally	1/64	1/128	1/64	1/128
	100	8.5	intravenously	1/16	1/16	1/64	1/256
			subcutaneously	1/128	1/128	1/128	1/256
			orally	1/64	1/128	1/32	1/64
P(Gly–Phe–Leu–Gly–Dox)(Gly–Phe–Leu–Gly–Gal)	300	21.9	intravenously	1/256	1/256	1/128	1/128
			subcutaneously	1/256	1/256	1/64	1/128
	100	7.3	orally	1/128	1/256	1/64	1/128
			intravenously	1/64	1/128	1/32	1/69
			subcutaneously	1/64	1/64	1/32	1/69
			orally	1/32	1/32	1/64	1/69
None (control)	—	—	—	1/32	1/32	1/16	1/16

Ten mice per group were immunized every third day (five times). On the third and sixth days after the last treatment the mice were exsanquinated and the sera stored at −70°C. Numbers represent an average of 10 individually tested sera.

and much more experimentation is needed to clarify this potential aspect of polymer activity.

Polymer–protein conjugates in clinical use

Soluble polymeric drug carriers are yet to become an established approach for cancer chemotherapy. However, it is important to emphasize that two soluble polymer-based systems have already shown important clinical activity. Both systems have been reviewed extensively by those principally involved in the programmes,[17,16] but their salient features will be briefly summarized here.

SMANCS

Maeda, Konno and colleagues have been responsible for the development of SMANCS.[17,41] This conjugate consists of two molecules of the synthetic polymer styrene-co-maleic acid/anhydride (SMA) (each of $M_w = 1500$) covalently bound to the antitumor protein neocarzinostatin (NCS) to give a final molecular weight of approximately 15 000 (Figure 18). The conjugate is soluble in organic solvents and the contrast agent lipiodol. Polymer conjugation was originally undertaken to increase the plasma half-life of NCS (a 10-fold increase was seen[148]) and to improve tumor and lymph node localization[76] (Figure 19), but additionally it has

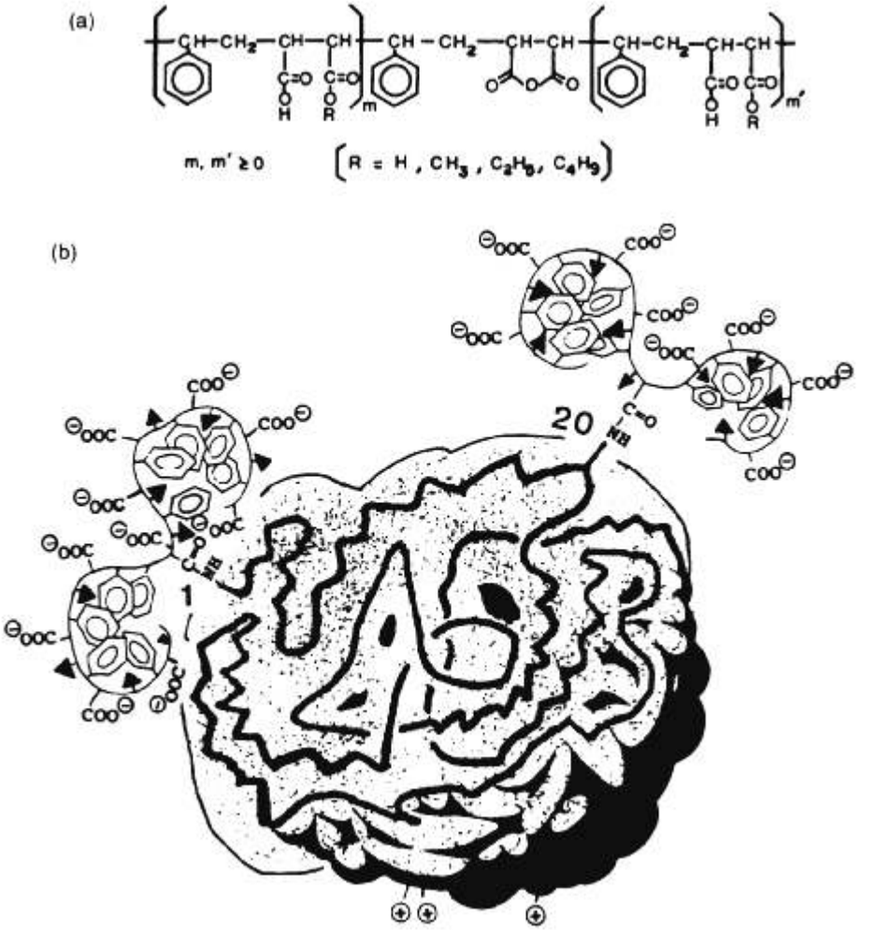


Figure 18. (a) Structure of poly(styrene-co-maleic acid/anhydride) and (b) diagrammatic structure of SMANCS. Note that two SMA molecules are attached to globular NCS at Ala_{-1} and Lys_{-20}, and the hydrophobic styrene and alkyl residues (shown as triangles) are clustered together in aqueous media. The plus signs indicate the positive charge of Arg in NCS (from Maeda *et al.*,[18] with permission).

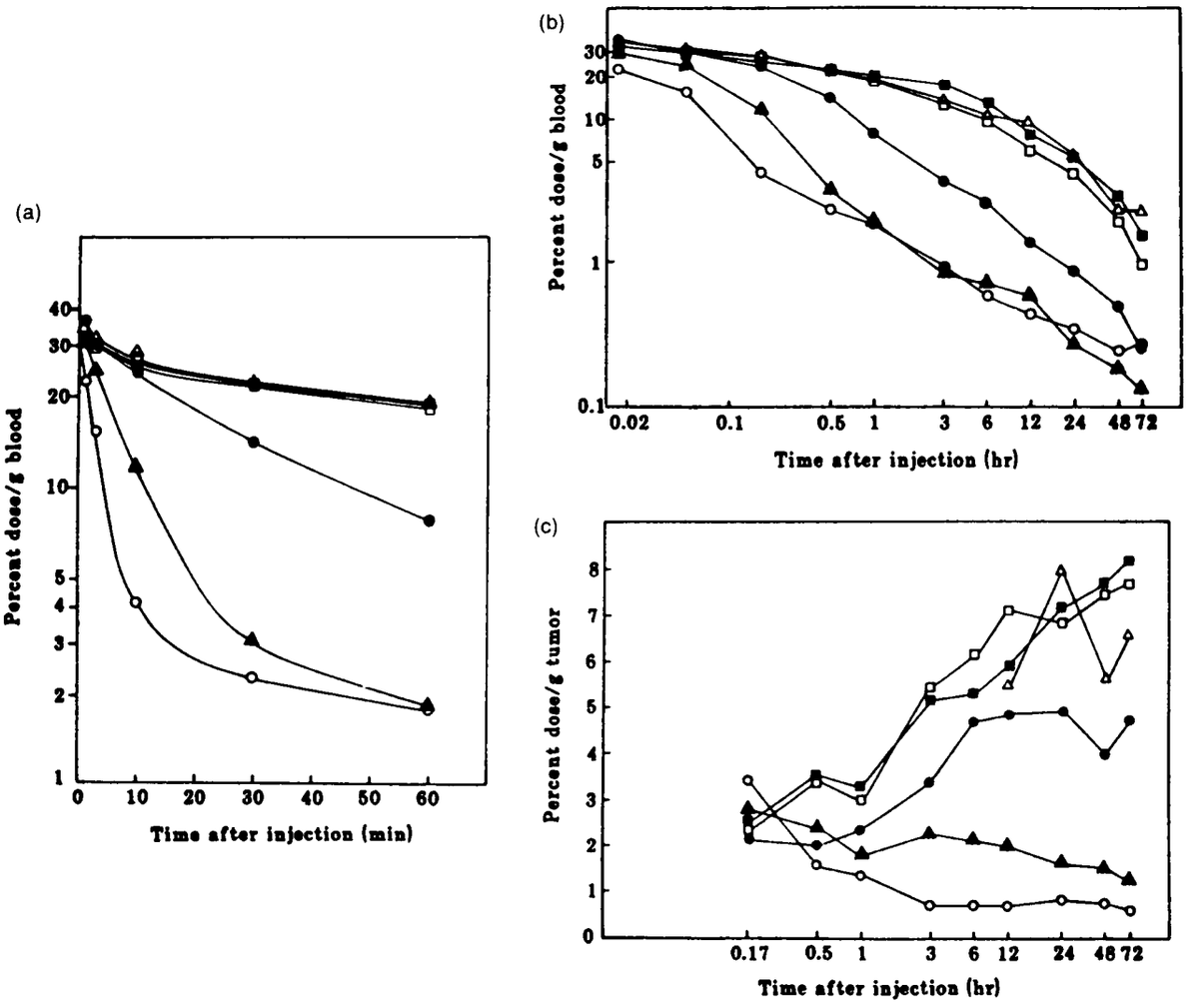


Figure 19. Plasma clearance and intratumor accumulation of various ^{51}Cr-tagged proteins in tumor-bearing mice. Plasma clearance of various proteins with molecular weights ranging from 12 000 to 160 000 during short and long time periods is shown in (A) and (B), respectively. Their intratumor concentration is shown in (C). ○, NCS (M_r 12 000); ●, SMANCS (M_r 16 000); ▲, ovomucoid (M_r 29 000); □, BSA (M_r 69 000); ■, mouse serum albumin; △, mouse IgG (M_r 160 000). Radioactivity proteins were injected intravenously at time zero. Values are based on radioactivity (from Matsumura and Maeda,[76] with permission).

since been shown that SMANCS causes macrophage activation[149] and stimulation of interferon production[150] and is generally immunostimulatory.[151] A tumor/blood ratio for SMANCS in lipiodol has been measured at above 2500 using a rabbit liver tumor model[152] which is much higher than reported for any other tumor targeted system.

SMANCS showed good antitumor activity in animal models and a number of clinical studies have now been reported which also show that this novel conjugate has remarkable antitumor activity. SMANCS is routinely administered in the lymphographic agent lipiodol via intra-arterial infusion by catheterization of the appropriate artery. X-ray monitoring ensures localized delivery. As this technique does not require major surgery administration can be carried out more or less on an out-patient basis and the procedure would be amenable to use in most hospitals. The first clinical evaluation of SMANCS was reported in 1983. Forty-four patients were selected, mostly with non-resectable hepatoma, and 86% showed decreased

α-fetoprotein levels and 95% showed a decrease in tumor size.[153] It was shown that 3–4 mg of SMANCS could be administered every 3–4 weeks and X-ray imaging confirmed selective retention of SMANCS in the tumor. In another pilot study, 24 patients with various solid tumors, including metastatic liver cancer, cancer of the gall bladder, lung and pancreas, were treated with SMANCS administered via the appropriate feeding artery.[154] Regression was seen in 13 of 18 evaluable patients, including six with metastatic liver cancer, one with unresectable gall bladder disease and in all four cases with low grade transient carcinoma of the lung. Figure 20 shows the changes in α-fetoprotein levels and tumor status seen in primary hepatoma patients treated with SMANCS.[41]

Subsequent and more rigorous evaluation has confirmed these early trends, and a multicenter phase II study with more than 200 patients with primary hepatoma has recently been completed in Japan. Preliminary data have been reported by Maeda.[17] For treatment of hepatoma the clinical dose of SMANCS (in lipiodol, 1 mg/ml) varies between 1 and 10 ml and is usually 3–5 ml. The tumor image is quantified using X-ray computed tomography (CT) and 0.25 mg of SMANCS (in lipiodol) administered per square centimeter of the cut surface area to ensure coverage of more than 50% (grade III) or 100% (grade IV) tumor image. Initially treatment has been administered monthly but this interval has been lengthened depending on the CT scan. The side-effects observed include low grade transient fever (50%) and some patients (about 20%) experienced dull abdominal pain, following hepatic artery administration for about 20 min, but this may result from angiography. No adverse side-effects were noted in lung, heart or brain. After intra-arterial administration a hematological effect is not usually observed, but slow intravenous infusion did, however, result in a decrease in white cell and platelet count, but this effect was reversible. The prognosis for patients with primary hepatoma depends on the presence of cirrhosis at time of diagnosis and the degree of tumor spread. Patients with no evidence of cirrhosis and tumor which has not spread to more than two segments of the liver have a 5 year survival of 90% when treated with SMANCS, exceptional activity if one considers the current prognosis to be 6 months (Figure 21).

SMANCS has also been used to treat metastatic liver cancer and renal cancer (drug is administered via the renal artery) but it seems that more frequent administration will be needed than now routinely used to treat hepatoma and also a high viscosity contrast agent containing a higher concentration of SMANCS (1.5–2.0 mg/ml) could to improve tumor localization and hence produce a response.

There appears to be several advantages of administration of SMANCS in lipiodol. Firstly, lipiodol is a contrast medium which can be used for lymphography, and therefore both the dose and the tumor image can be carefully quantitated by CT scan after intra-arterial administration. Secondly, it has been shown that extremely efficient tumor targeting can be achieved using this formulation. An aqueous formulation of SMANCS has been tested clinically in pilot studies against various solid tumors, and reported to be effective against ovary, esophageal, lung, stomach, adrenal and brain tumors, but not metastatic melanoma and metastatic bone tumors. In particular metastatic lung cancer, arising from primary kidney cancer showed an effective response rate of above 40% (Yamanaka and Kobayashi, unpublished results, reported in Maeda[17]). This formulation is currently in phase I and it is suggested that co-administration of a hypertensive agent such as angiotensin II may be needed to improve tumor accumulation of this aqueous form.[17]

PEG–protein conjugates

To date those soluble polymer conjugates most extensively studied in the clinic include mPEG covalently linked to proteins.[16,155] Protein modification with mPEG has been termed PEGNOLOGY℠ and several products have been developed by ENZON Inc. This polymer is approved by FDA for use as a vehicle or base in a number of pharmaceutical preparations and is available in a variety of molecular weights. It can be bound to the surface of proteins using several different coupling methods,[156] thus by varying the method of conjugation, the molecular weight of mPEG used and the degree of surface modification there is a possibility to control the nature and behavior of the resultant polymer conjugate. In general polymer conjugation increases the solubility and stability of a protein, increases the plasma half-time and markedly reduces its immunogenicity,[157] but unfortunately the biological activity of most proteins is also decreased following conjugation. It has been suggested that the increased plasma circulation time can more than compensate for this disadvantage. More than 30 proteins have been modified with mPEG[16] and their biological

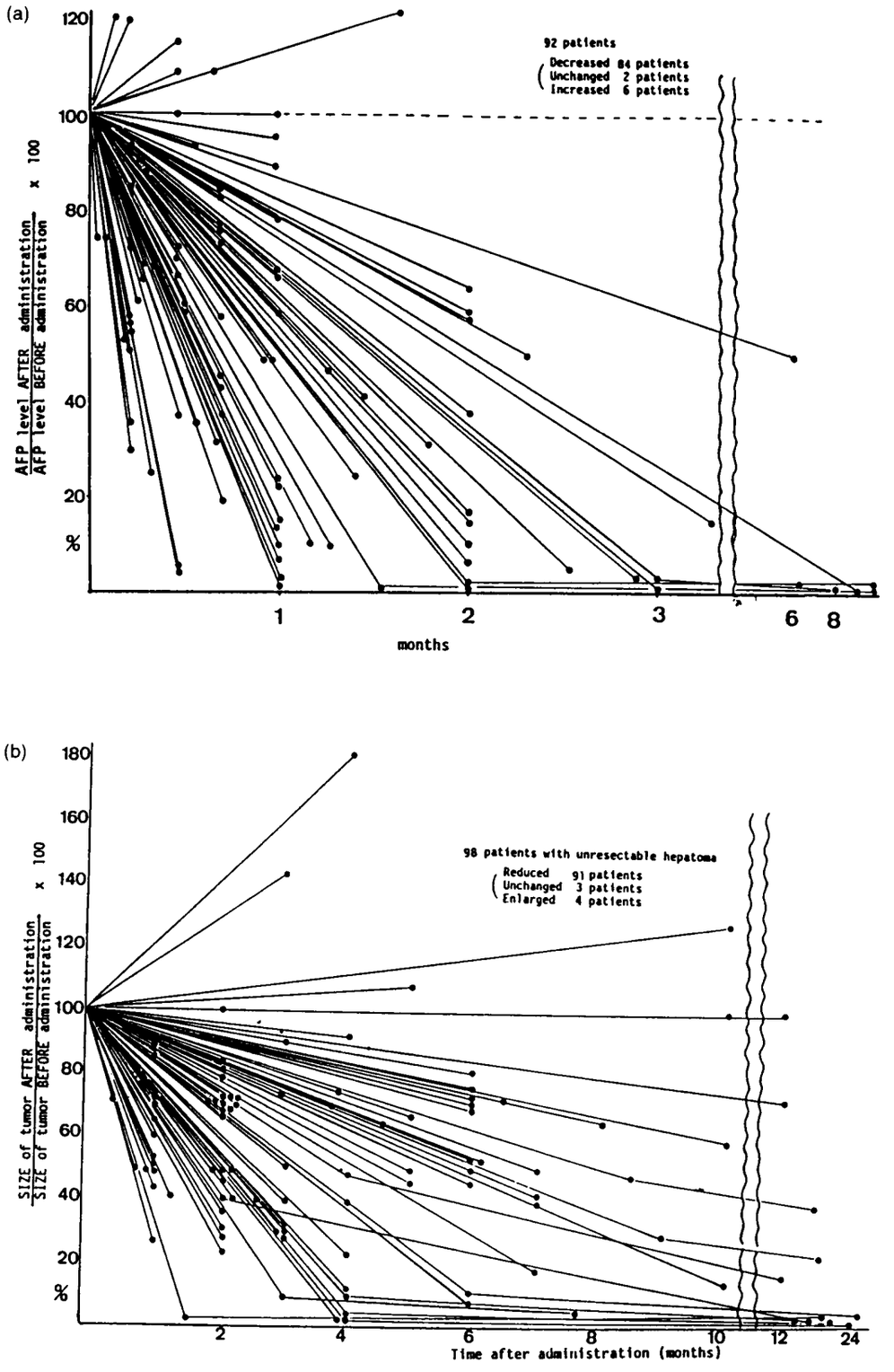
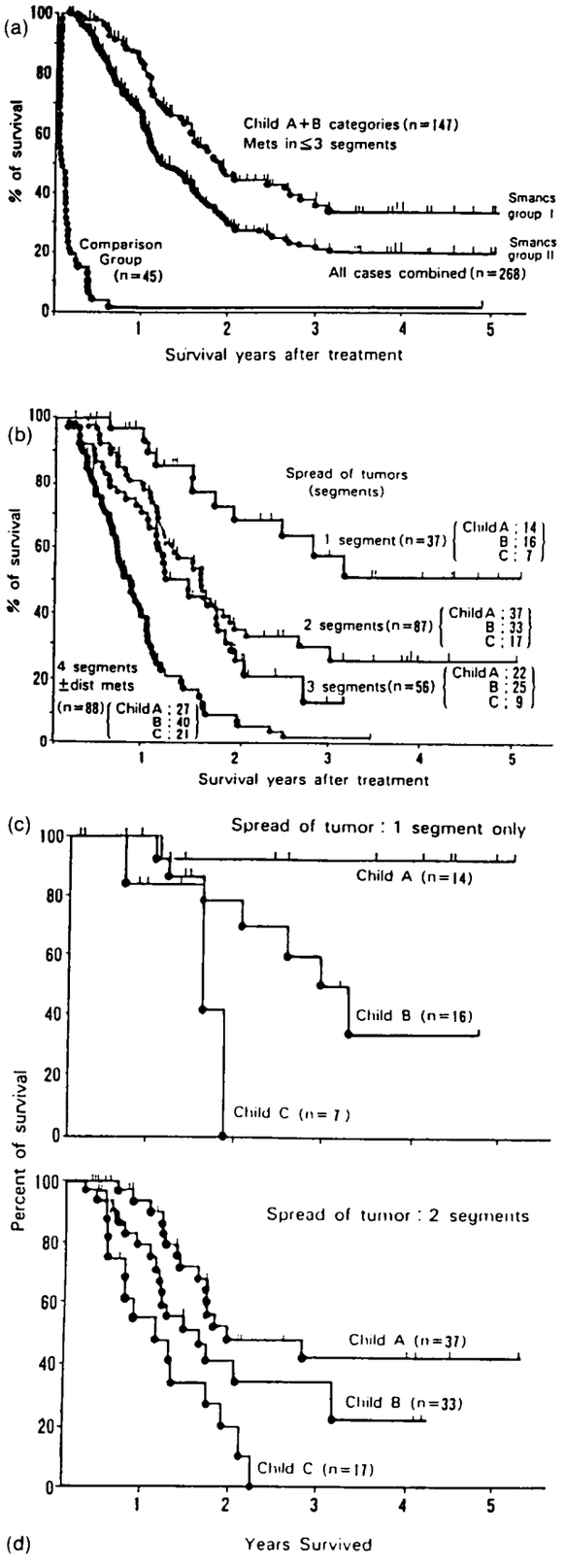


Figure 20. (a) Changes in tumor size after arterial administration of SMANCS/LPD in unresectable hepatocellular carcinoma (HCC). (b) Changes in α-fetoprotein value after administration of SMANCS/LPD in patients with HCC (from Konno and Maeda,[41] with permission).



properties followed. mPEG-modified adenosine deaminase (ADAGEN™) has already received approval for treatment of severe combined immunodeficiency disease associated with adenosine deaminase deficiency.

In the context of cancer chemotherapy, conjugates containing asparaginase, granulocyte colony stimulating factor (G-CSF) and IL-2 are the most relevant, but the ability of mPEG to reduce the immunogenicity of antibody conjugates and enhance their tumor localization could also prove very interesting. Asparaginase displays antitumor activity against acute lymphoblastic leukemia (ALL), and lymphomas, but it has been shown that the enzyme can induce mild allergic reactions, anaphylactic shock and hypersensitivity reactions, and host antibody production can lead to premature clearance of the enzyme from the circulation. In a phase I study involving 31 patients, of which 27 were pharmacokinetically evaluable, conjugation of mPEG to asparaginase increaased its plasma half-life in humans from 20 h (native enzyme) to 357 h (Table 12), and the volume of distribution indicated that like asparaginase the conjugate was mainly located in the plasma.[158] This adduct showed no hematological toxicity, no pancreatitis, but a transient increase in liver enzymes was seen. Reduced hypersensitivity reactions were experienced when using mPEG–asparaginase, but three anaphylatic reactions were observed, and in one case sudden disappearance of conjugate from plasma after a patient's third dose.

In a phase II trial involving patients with refractory non-Hodgkin's lymphoma treated intramuscularly with mPEG–asparaginase at a dose of 2000 units/m^2 every 2 weeks, partial response was observed in two of the 21 patients entered in the trial. Nausea and vomiting occurred in approximately half of the patients and was severe in nine, diarrhea and abdominal pain being seen in

Figure 21. Survival of patients with unresectable hepatocellular carcinoma (HCC) treated with intraarterial administration of SMANCS/Lipiodol. (A) Comparison of survival of patients after conventional therapy and after SMANCS/Lipiodol. (B) Survival of HCC patients classified according to metastatic spread of tumor in liver. (C) and (D) Survival of HCC patients according to the classification of tumor metastasis and the extent of liver cirrhosis (Child class A, B and C). All SMANCS-treated patients are inoperable and/or in advanced stage. Mets, metastasis. (Data: courtesy of Dr T Konno and SMANCS Pilot Study Group, Kumamoto University, reproduced from Maeda,[17] with permission).

Table 12. Clinical pharmacokinetics of PEG–L-asparaginase in 27 patients (from Ho *et al.*[158] with permission)

Drug	Dose (U/m^2)	$T_{1/2}$	V_d (ml/m^2)	AUC (U/ml day)	Cl_r (ml/m^2 day)
PEG–L-asparaginase	500	315	2111	5.2	99
	1000	317	1941	9.4	144
	2000	588	2553	27.1	77
	4000	184	1865	25.4	186
	8000	415	2143	89.9	117
mean		357	2093	10.2	128
L-Asparaginase	16 500	17	2146	7.3	2099
	50 000	20	2264	20.4	2174
	100 000	20	2881	32.8	2043
mean		20	2336	0.4	2196

AUC values were normalized to 1000 U/m^2 to present mean AUC value.

30% of cases, but there was absence of hematological toxicity which was suggested to offer the possibility of using the mPEG–asparaginase in combination with other chemotherapy.[42,159]

A number of preclinical studies have been reported that describe the synthesis,[160] pharmacokinetics[161] and biological activity of mPEG linked to biological response modifiers such as IL-2[162–164], and recombinant granulocyte colony stimulating factor (G-CSF).[165] As in the case of asparaginase, modification of IL-2 with mPEG prolonged the α and β plasma half-lives from 3 and 44 min to 48 and 310 min, respectively,[161] and decreased its immunogenicity in rabbits and mice,[162] the IL-2 specific IgG titres being 100- to 1000-fold lower. Clinical studies with this conjugate are underway.

Clinical potential

The disciplines of drug targeting and controlled release are still in their infancy, most preclinical experimentation being initiated in the last 15 years. As yet relatively few clinical trials have been reported, most describing liposomal formulations or immunoconjugates, with a smaller number of studies describing microparticulate systems for intra-arterial administration. Unfortunately most human studies have been pilot experiments with a small number of patients and often poorly characterized pharmaceutical formulations. Nonetheless, the emergence of Zoladex® as an approved controlled release formulation offers great encouragement. The current clinical experience with the broad range of targeted systems has recently been reviewed with the conclusion that no system has yet established itself as the treatment of choice for routine clinical use.[166] However, there is growing awareness that successful development of targeted systems will only be made if, like all the new drug delivery systems, they are manufactured to controlled and reproducible specification, and subsequently undergo rigorous phase I/II evaluation. Careful attention must be paid to the methods used for clinical pharmacokinetic analysis as the biodistribution, mechanism of drug release and hence bioavailability when administering a drug in the form of a delivery system is substantially different from that seen using conventional chemotherapy.

Already it has been shown that soluble polymeric carriers offer considerable promise. Clinical experience with SMANCS in Japan is without doubt encouraging and it seems vital that this approach is now tested more widely as a multicenter international trial. Ease of administration of the SMANCS conjugate does not preclude widespread use, but perhaps reluctance to develop this approach more widely has arisen from the uncertainty of the mechanism of action of the conjugate—a novel polymer coupled to an antitumor protein, NCS, administered in a lymphographic contrast agent. mPEG conjugates have broken the ice, establishing themselves as the first soluble polymer conjugates to achieve clinical use. Approval of mPEG–asparaginase would see the first antitumor product. The importance of soluble polymers as carriers for improvement of conventional chemotherapy has yet to be established, but one hopes that the interesting preclinical data obtained with the HPMA copolymer conjugates described earlier will be reproduced in the clinical situation. To the future, one could

speculate with optimism that there are on the horizon a whole new generation of polymeric systems for delivery of antitumor agents, including conjugated drugs, proteins and oligonucleotides. However, past experience with liposomes and immunoconjugates suggests the need for cautious optimism and much hard work!

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